Project No.
Book No.
TITLE SDS gel on 50ml Heparin

From Page No.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

I' DH10B/K2 mW 200 mW
P190 15λ 15λ

I' TF1 P181 200

Am SO4 resus 362 1/λ 1.5
5200 50 1/λ 105
Blue 62

Heparin frns
105 150
106 150
107 150
108 150
109 150
110 150
111 150
112 150
113 150
114 150
115 150

TF1 10+#TF310 10A-502
in/λ
H2O 100 100 - - 238 150 →
Vf = 300

15% TCA 300 → - - 300 →
(P72,9 and P507)
30 1/2

1x sample buffer 3550 µl 400 → 35

#4,5 sat (NH4)2SO4 TCA ppt

To Page N

Witnessed & Understood by me, Deeana Polys
Date 5/1/95
Invented by
Record d by
Dat 4-28-95

PAGE 5 OF NOTEBOOK WAS BLANK

From Pag No.

Buffer F, G as per rTaq 91342.PRP

make F, 4L first as follows.

make Vol up to ~~2700~~ 3200 ml (i.e. 80% of Vf of

remove 160 ml and add

20 ml Tween 20 (Pierce)

20 NP40 (Pierce)

ml 200

= buffer G

Take this remaining buffer up to Vf = 3200

for 1X buffer F

---

pool frm 105-114 of Heperin (P.1-4)

= 90 ml vol (actually measured 87 ml)

Dialyze against 2 buffer F, 5 hr

2 ml C O/N

4-30

Recovered 33 ml after Dialysis.

combine with 33 ml buffer G = Vf 66.

labeled: TF1 DNA pol in storage buffer 4-30-95 store at -20

4.33 U/l
ACP 8

To Pag N

Witn ssed & Understood by me,	Date	Inv nted by	Date
Deanna Robins	5/1/95	R c rd d by	4-29-95 / 4-30-95

Project No _____
6 Book No._____ TITLE Storage buffer

From Page No._____

Buffer F, G as per rTaq 9134Z.PRP

make F, 4L first as follows.

in flat Vol up to ~~3700~~ 3200 ml (ie 80% of Vf of

remove 160 ml and add
20 ml Tween 20 (Perce)
20 NP40 (Perce)
ml 200

= buffer G

Take this remaining buffer up to Vf = 3700
for 1X buffer F

---

pool frm 105-114 of Heparin (P.1-4)
= 90 ml vol (actually measured 87 ml)

Dialyze against 2 buffer F, 5 hr
2 ml C O/N

4-30

Recovered 33 ml after Dialysis-
combine with 33 ml buffer G = Vf 66

labeled: TfI DNA pol in storage buffer 4-30-95

store at -20

4.33 u/l
pol B

T Pag N

Witnessed & Understood by me,	Date	Invented by	Dat
Deanna Rowe	5/1/95	Recorded by	4-29-95 / 4-30-95

Unit assays for Tfi I

Pr j ct N .________

Book No.________

Exhibit **83**
Appl. No. 09/558,421

ge N .__	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	27
storage buffer (-30.9)																												
tion*																												
.50	2				2				2				2															
·00		2				2				2				2														
000			2				2				2				2													
:000				2				2				2				2												
t 30709B																												
5 (see P181)																												
50																	2											
00																		2										
00																			2									
100																				2								
310/0A-502																												
4-25-95 PA2,9																												
75 gel																												
50																					2							
100																						2						
00																							2					
400																								2				
4-20-95																												
SDS gel																												
50																									2			
00																										2		
00																											2	
100																												2
rxn mix (0.9)	48µl →																48 →											
unit assay mix													48 →															

74°C, 10', → 10µl 0.5M EDTA → spot 40µl on GFC

tubes # 13-16 use same serial dilution as tubes # 1-4
all 3 independent serial dilutions in Taq dil buffer

To Page No.______

ed & Understood by me,	Date	Invented by	Date
Veronica Polany	5/1/95	[signature]	5-2-95
		Recorded by	

[redacted]

Project No.______
Book No.______ TITLE ______

From Page No.__

	SAM	CPM1	pmol-200BKGD	u/µl		
dil 1	1	4167.00	102.8	3.85		
	2	2383.00		4.24	4.25 ave	
	3	1401.00		4.67		
	4	1060.00		(6.68)	→	
dil 2	5	4115.00		3.80		
	6	2316.00		4.1	4.38 ave →	4.33 u/λ
	7	1394.00		4.6		
	8	846.00		5.0		
dil 3	9	4180.00		3.9		
	10	2416.00		4.3		286,000
	11	1226.00		5.18	4.37 ave →	66 ml
	12	888.00		5.3		
Taq Rxn mix (500 pmol DNA)	13	4556.00		4.22		use of 500
	14	2720.00		4.89	4.66 ave ←	DNA substrate
	15	1452.00		4.86		Taq Rxn mix
	16	1330.00		(8.25)		w 80%
30709B	17	6287.00		1.18		unit w
	18	4373.00		1.62	1.6 u/λ ave	= 30%
	19	2428.00		1.73		total
	20	1302.00		1.87		units
1010A	21	13554.00		2.59		
	22	8692.00		3.5	3.5 u/λ	
	23	5151.00		3.8		
	24	3002.00		4.36		
1010A	25	12756.00		2.44		
	26	8623.00		3.27	3.51 u/λ	
	27	5397.00		4.04		
	28	2964.00		4.30		
BKGD =200	29	194.00				
	30	96542.00	57.9 cpm/pmol			

5-2-95 label Heparin fxn of 4-30-95 at

4.33 units/λ

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
Deborah a Polamp	5/15/95	[signature]	5-2-95
		Recorded by	

e N .__ follow-Taq QC: 10342.QCP

	(22 Rxns)	ss DNA Rxn mix	ds DNA Rxn mix	
CR buffer		110 µl	110 µl	✓
M Tris (@ pH 8.4)				
mM KCl				
mM $MgCl_2$		110	110	✓
74 (+) ssDNA 0.2µg/µl		110 µl		✓
174 RF 0.33 µg/µl			66.7	✓
@ H_2O		666 / 990	703.3 / 990	✓

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

-30.55	.46	.92	1.39	1.8-2.3												
u/µl																
micrin					.57	1.14	1.7	2.3	2.9							
OA-502																
µl (PB)																
rude ext																
I 4-13-95)																
K																
10,000							1									
1,000								1								
100									1							
10										1						
o dil											1					
O	4.5	4	3.6	3.15	2.7	4.X	3.9	3.3	2.7	2.1	4	4	4	4	4	5 – 5 ✓

il buffer

Vf=50µl 72°C, 3 hr (start 12:10 PM)

1 - 17 is 45µl ss ØX ONA Rxn mix and #18 - 34 is ds DNA mix 45µl Rxn mix

To Page No.____

ed & Understood by me, Blanco	Date 5/15/95	Inv nted by	Date 5-3-95
		R c rd d by	

[redacted]

Project No.________

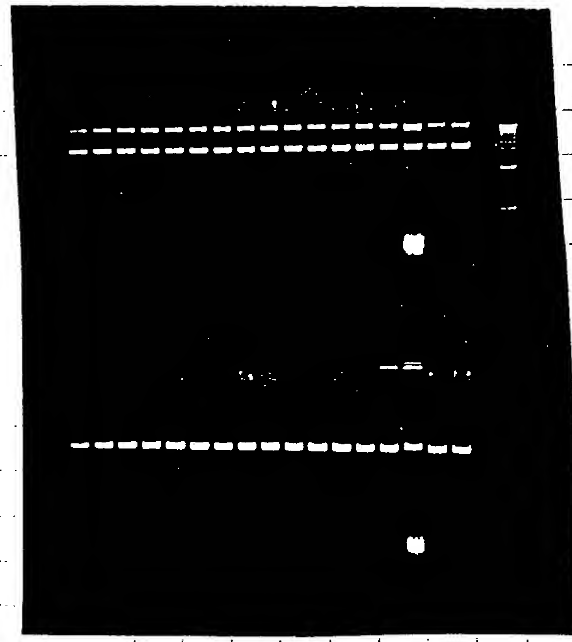
Book No.________ TITLE ____________________

From Page No.__

CTI Tf1 — Epicentre — crude ext / FrI

units 2 4 6 8 | 2 4 6 8 10 | Blank | dil buffer blank

(relaxed) RFII →
sc DNA →



} dsD endo

} ssD end

note for RF φx substrate with positive control (FrI) there is a slight increase in relaxed circles (ie nicked) and immediate conversion to small fragments. no nicking seen for either ssDNA or dsDNA substrates by any Tf1 or Epicentre.

To Page N

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	5/15/95	[signature] / Recorded by	5-3-95

Dilutions of Taq for PCR functional assay

ge No.

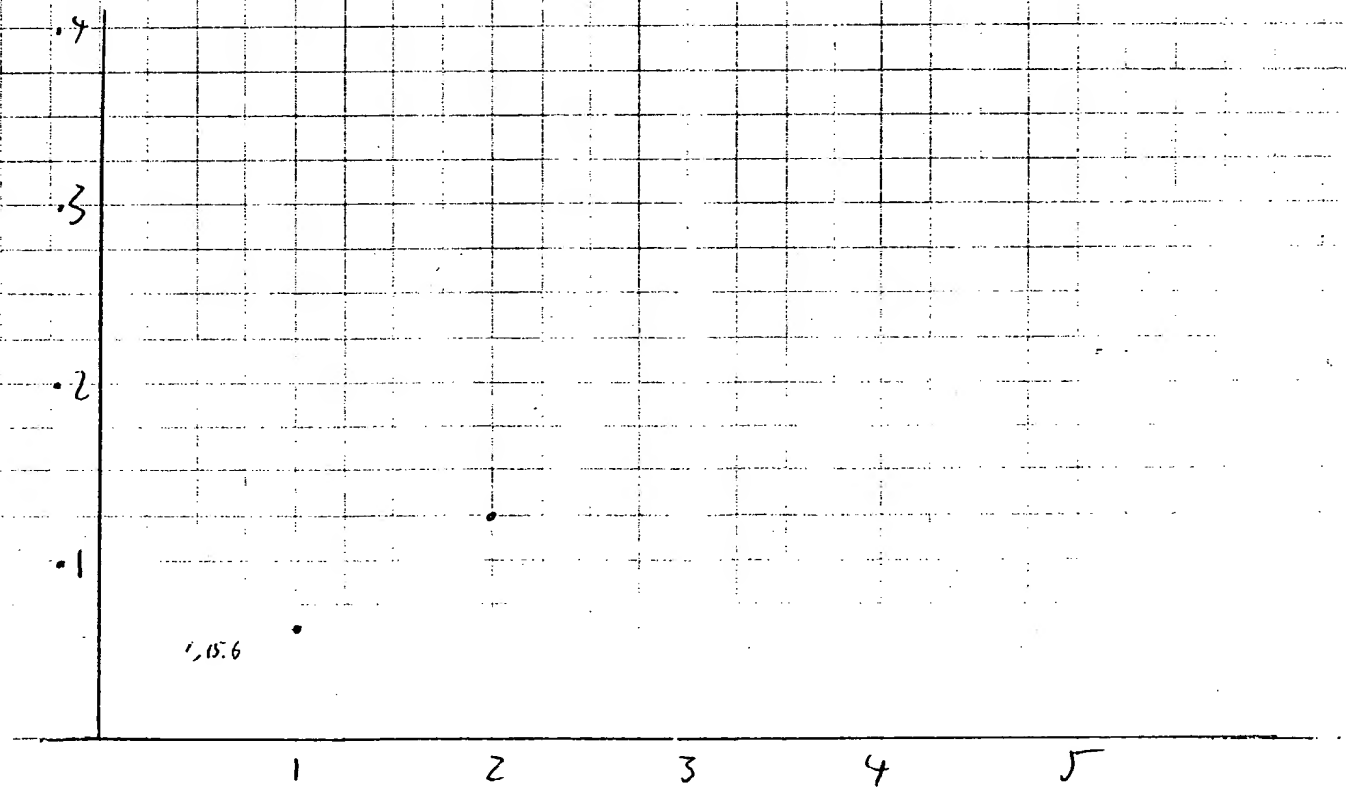
	#	1	2	3	4	5		1	2	3	4	5	
Cf u/μl		.0625	.125	.188	0.25	0.5							
Lot# EM7414		5	5	5	5	5		3	3	3	3	3	4
dilution buffer		395	195	128.3	95	45		237	117	77	57	27	36
Vf =		400	200	133.3	100	50		240	120	80	60	30	40

R401

	#	6	7	8	9	10

		11	12	13	14	15

plitaq



To Page No.

ed & Understood by me,	Date	Invented by	Date
Polano	5/15/95	R cord d by	

Exhibit **86**
Appl. No. 09/558,421

Project
Book No.______ TITLE 32P "33 correct" (as done P146,9 and 136,9) and at 5 µM primer Cf so 3' exo reaction can be Cf = 200 nM

From Page No.__

(P 131)
↓

H₂O 10.5

33 mer correct (P138,9)	20 pmol/λ (20 µM)	7.5	15 µl	✓ ✓	~ 300 pmol 33 mer
32P γATP 10 mCi/ml 5-12-95		5	30 µl	✓	11 × 10⁶ cpm/µl ~ 90
5 x kinase buffer		6	12 µl	✓	
PNK 10u/λ		1	3 µl	✓	
~~H₂O~~		30	60 µl	37°C, 30'	
				55°C, 5'	

Plan for fidelity assay for pol ± 3' exo

(33 correct P138,9) (–) dCTP (+) dA,T,G-TP

```
        ——————— CCAGTGAATT GAG T GGTA
———————————————— c TTAAGCTCGAGCCATGGGCCCC
```

same 5' ends as 23 mer on mp19+

n-1 is 3 nts downstream from primer 3' end

only 3 nts for rescue will have to do

most run times stopped here for quantitation at 49-51

To Page No.

Witnessed & Understood by me, [signature] Date 5/15/95

Invented by [signature] Date 5-9-95

Recorded by

unit assay on Tne
(in Tag kinit assay mix)

Pr ject N .
B ok No.

ge N

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
5 u/λ																			
.95																			
25	2																		
50		2																	
00			2																
00				2															
2000					2														
4000						2													
5-7-95																			
u/λ																			
1000						2													
2000							2												
4000								2											
7000									2										
16000										2									
32000											2								
4-30-95																			
u/λ																			
250												2							
500													2						
1000														2					
2000															2 — — —				

Sample	#	cpm	pmol	u	
Tne 3-2-95	1	3906.00	88.9	1.7	1.56
	2	2179.00			1.65
	3	1383.00			1.95
	4	929.00			
	5	916.00			−BKGD
	6	448.00			
Tne 5-7-95	7	8537.00	194	29.1	
	8	4263.00		29.1	33
	9	2648.00		36	
	10	1531.00		41	35
	11	776.00			
	12	570.00			
Tfl	13	4932.00	112	4.2	
	14	2336.00		4.0	
	15	1307.00			3.6
	16	1011.00			
	17	298.00	} BKGD → ave 239		
	18	266.00			
	19	153.00			
	20	105445.00	65.9 cpm/pmol		

conclude
Tne 3-2-95 ave 34 u/λ
(agrees with 36 u from Liz
Tne 3-2-95 1.72 u/λ ave

To Pag No.

d & Understo d by m , [signature] Date 5/15/95
Inv nted by [signature]
R c rded by
Date 5-5-95

From Page No.__ see P136,9 137,9 for procedure: 200 nM primer (
so its like a real PCR

	①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪	cocktail
10x PCR buffer 50 mM MgCl2	5	→								→		60λ ✓
34p 33mer correct" P12 (5 μM)	1.5μl	→								→		18λ ✓ (1.5 mM
H2O	2	→								→		24λ ✓
												102 vf - use 8.5 μl/
Tfl FrI 4-13-95	10											for #⑪
Tfl FrI'/PEI 4-13-95 (2.45 u/λ)		10										✓ 10 μl of 5x buffer
Tfl in storage buffer 4.33 u/λ (P8)			10									Nin (2-14-95 ou
												has dNTPs and
Tfl epicentre lot TF31010A-502 (3.5 u/λ P.7)				12.4								20 μl Tfl/vent
rTaq 5 u/λ EKBTI					10							0.745 λ from
												✓ 2 λ 34p33 a
The 3-2-95 1.72 u/λ (P.13) dilute to 0.5 u/λ						21.0						✓ 18λ H2O
												50λ vf
The 5-7-95 36 u/λ (according to Liz F and see P13 where I got 34 u/λ) dilute to 0.5 u/λ							21.0					
1% Tween20/NP40 H2O	0.5	0.5	1	2	1.5	3	1.5	3	1.5	3	1.5	✓
Taq dil buffer	31	31	31	29.1	31.5	29.5	31.5	29.5	31.5	29.5	10	✓

vf = 50x

74°C. remove 10 μl to 5 μl cycl seq stop
at 0.33, 1, 2 hr
run on 8% PAGE

see analyses of Tfl/vent epo rate and Tfl loss of full beg
33mer on P 46.

To Page N

Witnessed & Understood by me,	Date	Invented by	Date
	5/15/95	Recorded by	5-10-95

PAGE 15 OF NOTEBOOK WAS BLANK

Exhibit 88
Appl. No. 09/558,421

roject No. _____ ds 3'exo QC assay for Tf1
Book No. _____ TITLE 10342.QCP, 30042.SOP

From Page No. ___

	Mix A		
10 PCR buffer	110 µl	✓	Cf = 1X
50 mM $MgCl_2$	110 µl		Cf = 5 mM
3'(ds) substrate 0.5 pmol/λ lot EFE73	44 µl		(1 pmol/reacti
autoclaved, filtered H_2O	726 µl	✓	
	Vf = 990 µl		

acc to QC 103

units 0 2 4 6 8 10

tube #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
4-30-95 Tf1 (P.8 4.33 K/λ)	—	0.46	0.82	1.39	1.85	2.35									
Tf1 epicenter lot TF31N10A502 3.5 K/λ (acc P.8)	—								5.0	4.1	12.1	22.2	98.2		
Tf1 Fr I 4-13-95															

dil none 1/100
1/10 1/10
1/100 none

H_2O	3	9.5	4.1	3.6	3.2	2.7	5	4.4	3.9	3.3	2.7	2.1		✓
Tq storage buffer 2														✓
Mix A 45 µl	→													✓
Tq dil buffer									5	3	→			✓

Vf = 50 µl

74°C, 60' 37°C, 60'

put tubes on ice

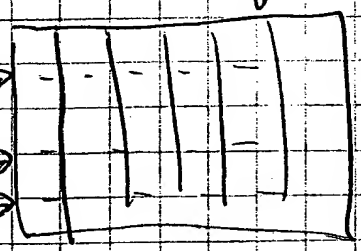
To Page N

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	5/15/95	[signature]	5-12-95
		Recorded by	

cut plate in half → get 20 × 10 cm plates

use 0.9 cm width lanes
origin at 2 cm

score lanes every 0.9 cm



spot 5 µl, dry, spot another 5 µl, dry

resolve in 2N HCl

bring solvent front to top of plate

Dry by heat lamps, not more than 7 min

count bottom = 3.2 cm (= ori)

3.2 cm – 7.6

7.6 = top

for each

do 5 × 18 = 17 tubes

add 3.5 ml fluor

count 3H

d & Understood by me,	Date 5/15/95	Invented by	Date
Dolan		Recorded by	5-12-95

[redacted]

Project No.________

Book No.________ TITLE To released

From Page No.__

Sample	Time	Tube	Counts	CPM	%
Tf1 4-30-95	0	1	4802.00		
		2	673.00		
		3	92.00	BKGD	—
	2	4	4782.00		
		5	709.00		
		6	123.00	31	0
	4	7	4935.00		
		8	702.00		
		9	114.00	22	0
	6	10	4670.00		
		11	743.00		
		12	129.00	37	0
	8	13	4732.00		
		14	669.00		
		15	123.00	31	0
	10	16	4788.00		
		17	767.00		
		18	123.00	31	0
Tf1 Epicentre	0	19	4661.00		
		20	613.00		
		21	84.00		—
	2	22	4677.00		
		23	636.00		
		24	145.00	53	0
	4	25	4185.00		
		26	767.00		
		27	140.00	48	0
	6	28	4586.00		
		29	774.00		
		30	112.00	20	0
	8	31	4136.00		
		32	887.00		
		33	111.00	19	0
	10	34	4202.00		
		35	793.00		
		36	97.00	1	0
Tf1 FrI	.02λ	37	1404.00		
		38	396.00		
		39	1801.00	1709	50%
	0.2λ	40	70.00		
		41	133.00		
		42	2536.00	2461	93%
	2λ	43	27.00		
		44	208.00		
		45	2466.00	2374	91%

Concluded no ds 3' exo activity is detected in either LTI or Epicentre purified Tf1 pol

To Page No

Witnessed & Understood by me, [signature]

Date 5/15/95

Invented by [signature]

Recorded by

Date 5-12-95

Adjust Tfl storage buffer from Taq to Epicentre Tfl conditions Project N

B ok No.

ge No.

	Epicentre Tfl storage buffer	LTI rTaq storage buffer	
Tris HCl	50mM pH 7.6 (pH at Room Temp based on Tele call to Epicentre Tech line)	20mM pH 8	need to increase molarity from 20mM to 50mM and pH from 8 down to 7.6
NaCl	100mM	0	need to add 100mM NaCl to LTI SB
glycerol	50%	"	LTI same as epicentre
DTT	1mM	"	
EDTA	0.1mM	"	
Tween 20/NP40	0.5% each	"	

Experiment	1M Tris HCl	pH (room Temp)	
10ml buffer G	0	8.02	(expect 8.0)
	5 λ	7.99	add 125 μl of 1MTris HCl to 10ml Tfl 4-30-95
	20 λ	7.92	
	20 λ	7.81	
	20 λ	7.77	
	20 λ	7.72	
	20 λ 20 λ	7.67	

Cf = 20mM Tris (in SB)
+ 12.5 mM Tris HCl added
32.5 mM

will follow this exact procedure for 10ml of Tfl 4-30-95
(P.8 4.33u/λ ⇒ new Cf = 10.0/10.614 4.33 = 4.08u/λ)

add 182 μl 1MTris pH 7.5 ⇒ Cf = 50mM pH = 7.60 pH

Vf = 182μl + 125μl = 307μl
plus 307μl glycerol
Vtotal = 10.614 ml
plus 62mg NaCl (FMW) Cf = 100mM

mix end over end ✓ 30 60 min

To Page No.

d & Understood by me, Bolcups | Date 5/15/95 | Inv nt d by [signature] | Date 5-12-95

R cord d by

From Page No. follow p. 84,9

[A]

5x Cheng (no dNTPs)	200 µl	✓
H_2O	637.9	✓
activated DNA 3.7 mg/µl	135.1	✓
dATG-C-TP 10 mM ea	5 µl	✓
^{32}P 2' dATP 10 mCi/ml 5-19-55 w/ date 3000 Ci/mmol	2 µl	

V_f = 750 µl for 10 Rxns

Cheng 1X
glycerol
Tricine pH 9.0 20
KOAc. 85
DMSO 20
$MgOAc$ 1.05
0.5 mg/
Cf 50 µM

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨

[A] 98 µl →

TF1 LTISB +Vent (5-16-55) 1.89 µl →

TF1 Epicenter SB +Vent (5-16-55) 2 →

TF1 Epicenter Enzyme + Vent (5-16-55) 1.95 µl →

V_f = 101 µl

68 °C, remove 10 µl to 5 µl 0.2M EDT (spot 10 µl on 6-FC), and remove 5 µl to 5 µl Kill solution with cold dATP (P84,9) and spot 2 µl on PEI (P84,

at 0, 10, 20, 40 min

Replate 1 on P. 40

Conclusion: There is no real good way to do this experiment because Vent is present at very low level compared to ∴ incorporation is saturated at levels where turnover is barely detected by PEI method. Will have to settle dsDNA 3' exo QC assay for units: see P 26

To Page

Witnessed & Understood by me,	Date	Invented by	Date
Dennis Doyle	6/9/95	Rec'rd by	5-11-55

Pr j ct N .
Bo k N .

Turnover

ge No.
.247.00
3736.00
4880.00
6046.00
2170.00
3243.00
3897.00
5694.00
2774.00
3405.00
4090.00
6613.00
3451.00
5498.00
6034.00
3820.00
2169.00
2604.00
2612.00
580.00
2193.00
2755.00
480.00
542.00
053.00
113.00
817.00
182.00

Turnover

Experiment 17

29	1925.00
30	3149.00
31	2983.00
32	3230.00
33	1091.00
34	1772.00
35	3000.00
36	3722.00

microp

		pmol
1	68601.00	1487
2	118176.00	2562
3	155582.00	5372
4	95344.00	
5	125044.00	
6	138326.00	
7	93269.00	
8	119376.00	
9	167655.00	
10	93777.00	
11	116666.00	
12	131003.00	
13	109619.00	
14	126936.00	
15	143456.00	
16	90599.00	
17	103792.00	
18	162204.00	
19	96493.00	
20	124924.00	
21	162506.00	
22	91166.00	
23	125191.00	
24	167630.00	
25	84292.00	
26	127063.00	
27	153977.00	
28	2135.00	BKGD
29	975.00	BKGD
2λ 30	110991.00	
2λ 31	110539.00	ave 110765

69.2 cpm/pmol

To Page No.

ed & Understood by me,	Date	Invented by	Date
[signature]	5/9/85	[signature]	5-16-85
		Recorded by	

[redacted]

Project No.________ Book No.________ TITLE S200 of Tne

Exhibit **90**
Appl. No. 09/558,421

From Page No. __

* buffer "S"

			Cf
1M Tris HCl 7.5 pH	20 ml	✓	20 mM
0.5 M EDTA	0.2 ml	✓	0.1 mM
glycerol	80 ml	✓	8 %
β me	0.357	✓	5 mM
3M KCl	16.7 ml	✓	50 mM
	1 L		

poured 6 ml S200

~~wash~~ equilibrate at 1 col vol/hr
(= 0.1 ml/min) for 2 hr

load 120 µl of Tne 36 u/µl 5-7-95
(= 2% col vol) (4320 units total)
Loaded on S200
by gravity.
elute at 1 col vol/hr
~~into ~ 95 fr~~

collect 50 µl frns (30 sec/fr
span ~0.5 A (??)

* note no detergent in buffer S
maybe this is why activity died after a
few weeks at 4°C (see P53)

To Page

Witnessed & Understood by me, [signature]
Date 6/9/95
Invented by [signature]
Date 5-17-95
Recorded by

Exhibit 91
Appl. No. 09/558,421

24 Project No. _____ Book No. _____ TITLE Assay 5200 fractions

From Page No. _____

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

fr # 10 13 16 19 22 25 28 30 35 40 45 50 55 60 65 70

Dilute 1/100 →

2 µl →

Top Rxn mix 48 →

pH 8.5 10, 74

Results from Top of p. 25

Frm		cpm	pmol	µ/µl
10	1	9978.00	199	3.0
13	2	9430.00		
16	3	16574.00		
19	4	12045.00		
22	5	10155.00		
25	6	7256.00	2.2	
28	7	6430.00	1.9	
30	8	6091.00	1.8	
35	9	3680.00	1.1	
40	10	2746.00	0.82	
45	11	2002.00	0.60	
50	12	1476.00	0.44	
55	13	1118.00	0.33	
60	14	694.00	0.2	
65	15	970.00		
70	16	603.00		
~~200~~	17	120535.00	75 cpm/pmol	

tr			µ/µl
3	1	3216.00	0.97
4	2	3990.00	1.2
5	3	5279.00	1.6
6	4	5148.00	1.55
7	5	6128.00	1.8
8	6	8950.00	2.7
9	7	11386.00	3.4
10	8	16350.00	4.9
11	9	14464.00	4.3
12	10	19127.00	5.8
13	11	23242.00	7.0
14	12	24609.00	7.4
15	13	25276.00	7.6
16	14	24319.00	7.3
17	15	23374.00	7.0
18	16	16929.00	5.1
19	17	14764.00	4.4
20	18	15075.00	4.5
21	19	13028.00	3.9
22	20	10781.00	3.2

To Pag 1

Witnessed & Understood by me, [signature]

Date 6/9/95

Inv nted by [signature]

R cord d by

Dat 5-12-15

N ___ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 -

1/150

Results on P 24

1-95

unit assay on fr 13-17 : pool 20 µl of each

13-17 1 2 3 4 5 6 7 8 9 10

100 2
200 2
400 2
800 2
1600 2
3200 2

7-95 3 u/λ
1000 2
2000 2
4000 2
8000 2

BIOS 11 13 1 183.00
12 14 1 216.00
2 Rxn mix 13 15 1 115777.77 72%

10,740

			pmol	u/µl
3	1	16254.83	33	5.1
4	1	12439.50		7.8
5	1	5518.00		6.9
6	1	2909.00		7.5
7	1	1741.00		8.2
8	1	866.00		
9	1	17122.03	35	53.2
10	1	10433.3	44	9.1
11	1	5683.00	110	70
12	1	3137.00	27	7.7

ave 7.7 u/λ

70.7 ave
agrees with 3b (see P13)

well normalized 5200
Pool 13-17 against 36 u/λ
value for Tue 7-7-95
(see P 25) 200

pool 13-17 (7.7 / 36 u/λ)(70.7)
= 3.9 u/λ

(3.9 u/λ)(7 rxns)(5 µl/rxn)
135 units received.

To Pag No.____

d & Understood by m ,
Blank

Date 6/9/95

Invented by

R corded by

Date 5-18-95
5-19-95

ig N .__

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

1/100

Results on p 24

9-95
innit assay on fr 13-17 : pool 20 µl of each

	1	2	3	4	5	6	7	8	9	10
13-17			1	2	3	4	5	6	7	8	9	10
1/100			2							
1/200				2						
1/400					2					
1/800						2				
1/1600							2			
1/3200								2		

5-7-95 3 u/l

1/1000								2		
1/2000									2	
1/4000										2
1/8000										

			pmol	u/µl
3	1	16254.83	33	5.1
4	1	12439.50		7.8
5	1	5518.00		6.9
6	1	2909.00		7.5
7	1	1741.00		7.7
8	1	866.00		
9	1	17122.03	55	53.2
10	1	10433.33	40	70
11	1	5683.00	40	77
12	1	3137.00		

ave 7.7 u/l

70.7 ave
agrees with 3b (see p 13)

BIG25 Remix

11	13	1	183.00
12	14	1	216.00
13	15	1	115777.77

10,740

well normalized 5200
Pool 13-17 against 36 u/l
value for Tue 5-9-95
(see p 13) 00

pool 13-17 $\frac{(7.7)(36\ u/l)}{70.7} = 3.9\ u/l$

(3.9 u/l)(7 rxns)(5 µl/rxn)
1365 units received

To Pag No.____

sed & Underst od by me,	Date	Invent d by	Date
Polanp	6/9/95	Record d by	5-18-95 5-19-95

Project No.

26 Book No._____ TITLE 3' exp (QC) assay for Tf1/vent

From Page No.__ (see P20 where turnover on PEI dish 2 wells)

follow assay on P 16-17

actual units of Vent added based on 0.09 u vent/0.94 TA/u

0 1 2 3 4 5 6

Mix A P.16 4545 →

Tf1/vent LTESB (5-16-95) 2 2 2 2

1/10	.02
1/100	.002
1/1000	.0002
1/10 000	.00002
1/100 000	.000002

Tf1/vent Equiv SB (5-16-95)

1/10
1/100
1/1000
1/10 000
1/100 000

Tf1/vent with equiv Tf1 (5-16-95)

1/10
1/100
1/1000
1/10 000
1/100 000

H_2O 3 →

68°C, 60 min

BKGD → 88.00 21.31 = BKGD start 2:35 pm

no dil	1	1684.00	4.87	1596
1/10	2	1323.00	5.49	1255
1/100	3	448.00	9.44	360
1/1000	4	143.00	16.71	55
1/10 000	5	96.00	20.39	8
1/100 000	6	101.00	19.90	13

Result: assay is not very linear in any range. Will assay at 1/10 and 1/100 in triplicate dilutions

T Page N

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/9/95	[signature]	5-22-95
		Recorded by	

[redacted]

Exhibit 94
Appl. No. 09/558,421

Project No.______ Benelta
Book No.______ TITLE 2 µl of enzyme dilution

From Page No.___

Slum avl of Triplicates

% lost from ori

minus BKGD

Front middle ori

Front middle ori	413.00	4406				282.00	avl
	441.00					681.00	
	3550.00				Vent	3216.00	
I SB	345.00	4050	314	244		252.00	3193 ori
	557.00		520	199		623.00	
	3148.00		3275	-755	19%	3170.00	Slum
	185.00	3874			BKGD	85.00	4177
	562.00					325.00	
	3127.00					3767.00	
duplicate SB	308.00	4410			BKGD	60.00	4669
	524.00					317.00	
	3578.00					4292.00	
	272.00	4216	302	229		469.00	
	603.00		532	231		271.00	
	3341.00		3434	-596	15%	4391.00	
	325.00						
	528.00						
	3383.00						
duplicate Tf1	412.00						
	509.00						
	2885.00						
	453.00		410	387			
	515.00		486	115			
	3004.00		2995	-1035	26%		
	515.00						
	434.00						
	3096.00						

$$\frac{(4030-2995)}{4030}$$

To Pag N

Witn ss d & Und rst d by me, [signature]

Date 6/2/95

Inv nted by [signature]

Dat 5-23-95

R c rded by

Result
5λ enzyme dil

g N ____

		TIME		total sum	% lost from ori
1	front	324.00			
2	middle	726.00	1.00		
3	ori	1780.00	1.00	2830	
4	ave	348.00	1.00		
5		960.00	1.00	3117	
6		1809.00	1.00		(43%)
7		458.00	1.00		
8		830.00	1.00	2991	
9	1764	1703.00	1.00		
10		483.00	1.00		
11		608.00	1.00		
12		1726.00	1.00	2817	
13		477.00	1.00		
14		515.00	1.00		
15		1698.00	1.00	2690	
16		288.00	1.00		(46%)
17		856.00	1.00		
18	1658	1670.00	1.00		
19		729.00	1.00		
20		627.00	1.00		
21		1452.00	1.00		(57%)
22		632.00	1.00		
23		511.00	1.00		
24		1199.00	1.00		
25		471.00	1.00		
26		686.00	1.00		
27	1354	1410.00	1.00		
28		374.00	1.00		
29		664.00	1.00		
30		1398.00	1.00		(50%)
31		200.00	1.00		
32		786.00	1.00		
33		1641.00	1.00		
34		128.00	1.00		
35		947.00	1.00		
36	1573	1682.00	1.00		
37		97.00	1.00		
38		477.00	1.00		
39		3007.00	1.00	3581	
40		66.00	1.00		
41		591.00	1.00		
42		3146.00	1.00	3803	
43		60.00	1.00		
44		480.00	1.00		
45	3122	3214.00	1.00	3754	
			1.00		

Result. The sum of all counts when enzyme is present (ave 2879) is less than for the no enzyme blank (ave 3713 ≈ 22% of counts unaccounted for.

43% in each case ≈ 1500 CPM is lost from origin (ie ^3H removed by exo) but ≈ 1/2 of that appears in front and middle. Also, why does any appear in middle it looks like ^3H MP partly missing in middle.

since ^3H runs in front it may be quenched by DNA contaminants that smear PET at the front.

% label removed from ds DNA substrate

	2λ enzyme mix	5λ enzyme mix
CTI SB	19%	43%
Epicentre SB	15	46
Epicentre TFI	26	57
Vent pol	21	50

To Page No. ____

d & Understood by me,
Date 6/9/95
Invented by
Recorded by
Date 5-24-95

Project No.
Book No.
TITLE Pol unit assay for Nini's Tf1/Vent m
same as P20 except need less units
[illegible]
" as for Taq unit assay P 12, 9

From Page No.

" Tf1 Chem unit assay mix

H_2O	1,056 ml	✓✓✓	35 Rxns
5X Chem buffer (see P 20)	350 λ	✓✓✓	
activated DNA 3.7 mg/ml	236 λ	✓✓✓	(0.5 mg/ml)
dATCG-TP 10 mM ea	35 λ	✓✓✓	200 μM each)
^{32}P α dCTP 10 mCi/ml	3.5 μl λ	✓	
	1.68 ml		use 48 μl/Rxn

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Tf1 Chem unit assay mix	48 μl →																							
used "Tf1 4" " #4 working stock																								
Tf1 4.33 u/λ (P8)																								
1/250	2				2																			
1/500		2				2																		
1/1000			2				2																	
1/2000				2				2																
Tf1/Vent 5/16/95 LTISB (~1u/λ Tf1 units)																								
1/250									2				2											
1/500										2				2										
1/1000											2				2									
1/2000												2				2								
Tf1 vent 5/16/95 Epicentre SB																								
1/250																	2				2			
1/500																		2				2		
1/1000																			2				2	
1/2000																				2				2
Tf1/Vent 5/11/95 (Epicentre Tf1)																								
1/250																								
1/500																								
1/1000																								
1/2000																								

} using duplicate serial dilutions for each set

(Tf1/Vent 5/16/95 LTISB:) assuming Epicentre units its ~4u/λ using my units (P.8) for Tf1

~~[illegible]~~ 74°C, 10'

dilute by adding 2λ enzyme to 498 μl Taq dil buffer for

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/9/95	[signature]	5-24-95
		Recorded by	

(-DVGD)

	SAM	CPM1	pmol	u/µl
TFI	1	4165.00	127	4.77
	2	2395.00		5.17
	3	1575.00		6.22
	4	1018.00		6.9
	5	4108.00		4.70
	6	3019.00		6.7
	7	2232.00		9.5
	8	1205.00		7.7
LTI SB	9	3788.00		4.5
	10	2354.00		5.0
	11	1425.00		5.47
	12	1090.00		7.62
	13	4135.00		4.7
	14	2353.00		5.0
	15	1688.00		6.78
	16	1095.00		7.67
Epicanth SB	17	3543.00		4.00
	18	2336.00		5.00
	19	1431.00		5.51
	20	1040.00		7.13
	21	3388.00		3.81
	22	2191.00		4.64
	23	1354.00		5.12
	24	857.00		7.31
Epicanth TFI	25	3746.00		
	26	2188.00		
	27	1548.00		
	28	866.00		
	29	4053.00		
	30	2474.00	71	5.3
	31	1456.00		
	32	905.00		
B/CGD	33	322.00		
2λ Rx min	34	72445.00	45.3 CPM/pmol	

(was 4.33 u/λ on P8)

will repeat this with 5 duplicates of the 1:50 pl dil for each on P.47

To Page No.

d & Understood by me,	Date	Invented by	Date
Solano	6/9/95		5-24-95
		R corded by	

Exhibit **96**
Appl. No. 09/558,421

Project No. ______ contents: 1.1x field test ("old")
assay samples P121,9 Book No. ______ TITLE new, BandM 2X mix

From Page No. 0

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
rTaq 1-31-95																									
5u/µl																									
1/125	2			2																					
1/250		2			2																				
1/500			2			2																			
1.1X new (may 8, 1995																									
no dil							2			2															
1/2								2			2														
1/4									2			2													
1.1x old																									
no dil													2			2									
1/2														2			2								
1/4															2			2							
BM 2X																									
1/2																			2			2			
1/4																				2			2		
1/8																					2			2	—

Taq rxn mix (P120,9) 48 µl / 50 µl → 74°C, 10'

see circled #a P35

for 2x Taq/125 and 2 µl undiluted of sample

$$\frac{\text{new } 6506 + 7030}{\text{rTaq } 10692 + 12403}(.04 \text{ u/}\Delta\text{ Taq by def}) = .023$$

$$\frac{6729 + 6660}{10692 + 12403} \quad .023$$

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/9/95	[signature]	5-25-95
		Recorded by	

g No.

			pmol	u/µl			
1/125	1	10692.00	417	7.7			
1/125	2	5333.00		7.7			
1/125	3	3112.00		8.0	← (-333 BKGD)		
	4	12403.00		8.9			
	5	6387.00		9.2	Total units in	* in 50 µl at 1X	average
	6	3853.00		10.0	in 50 µl at 1.1X		
mod dil	7	6505.00	250	.038 u/µl	1.88 u	1.71	* 1.76
1/2	8	4314.00		.050	2.50	2.27	
1/4	9	2381.00		.047	2.39	2.27	2.22
	10	7000.00		.040	2.0	1.82	
	11	4401.00		.051	2.5	2.27	
	12	2364.00		.047	2.4	2.1	* 1.75
	13	6729.00		.039	1.95	1.77	
	14	3962.00		.046	2.3		1.88
	15	2247.00		.045	2.2		
	16	6660.00		.038	1.90	1.73	
	17	3659.00		.038	1.9		
	18	1940.00		.037	1.9		
1/2	19	3456.00		.036	.87		
1/4	20	1705.00		.032	.77		0.86
1/8	21	1368.00		.047			
1/2	22	3028.00	117	.051	.84		
1/4	23	2005.00		.039	.94		
1/8	24	900.00		.026			
	25	333.00	BKGD				
	26	62215.00	37.9 cpm/pmol				

7.7 is a reflect since this "5 u/µl" is normalized to amplitude that was ~8 u/µl

red is average
tal units in 50 µl
d 1X for the assays of
undiluted/µl of 1.1X mix
e use 1.76 units for "new"
d 1.75 units for old "field test"
as my first time points for these samples. (note: appx ~2
ed dilutions above indicate 2.22 u and 1.89 u for old and new)
see next assay on P52 which is 1 month time point
using 2 µl undiluted.

* mix is 1.1 X
so at 1X mix is
(u/µl in 1.1X)

To Page No.

ed & Understood by me,	Date	Invented by	Dat
Polano	6/9/95	Record d by	1/25/95

Exhibit 97
Appl. No. 09/558,421

Project No.________ Book No.________

TITLE *unit assay for stability assay of 1,1X mix (p121, 9) (at*

From Page No.__

Group	Well	Counts	ave U/µl	% of p.122 (zero time p...)
1	1	8878.00	.036	98
	2	9039.00		
	3	9623.00		
2	4	8220.00	.032	98
	5	8228.00		
	6	8109.00		
3	7	8855.00	.034	106
	8	8307.00		
	9	8584.00		
4	10	6857.00	.027	93
	11	7096.00		
	12	6660.00		
5	13	9295.00	.035	105
	14	8535.00		
	15	8519.00		
6	16	6780.00	.026	98
	17	5930.00		
	18	5879.00		
7	19	8250.00	.034	104
	20	8545.00		
	21	9288.00		
8	22	8605.00	.032	91
	23	7590.00		
	24	7975.00		
9	25	7909.00	.031	99
	26	7993.00		
	27	7301.00		
no added 10	28	2863.00	.012 u/l; data from p 37, 38 → .013	
	29	3151.00		
	30	3188.00		
11	31	7926.00	.030	94
	32	7626.00		
	33	7355.00		
12	34	8180.00	.034	100
	35	8930.00		
	36	9000.00		
13	37	2662.00	.011	35
	38	2885.00		
	39	2632.00		
14	40	8091.00	.031	89
	41	7872.00		
	42	7722.00		
15	43	7664.00	.031	97
	44	7828.00		
	45	8063.00		
rTaq	46	10091.00		
	47	9701.00		
	48	10062.00		
	49	10476.00		
	50	10230.00		
	51	101.00		
	52	56411.00		
	53	57488.00		

(repeat w/ del in f... p 153) ← group 10

will repeat ← group 13

see new results on p...

10117 ave ⇒ 42 bpmol ⇒ 7.99 units/µl ...
(expect ... 5 u/l EKBTI ... normalized to amplitaq of ...)

(35.6 cpm/pmol) → wells 52, 53

To Page No.

Witnessed & Understood by me, *D Polap*   Date 6/9/95

Invented by *[signature]*   Date 5-26-95

Recorded by   5-26-95 *[initials]*

Stability of 1:1 X at room temp Project No. _____ Book No. _____

g No.__

0 time on P.154, 9 3-13-95
1 month P.174, 9 4-11-95

assay same as P.121, 9 for 4°C stability study
used same assay mix as P 5-25-95

Temp Rx #	Reaction Tube #	µl enzyme Rxn	1% Tween 20 NP40	Tag mix assay mix (P.121,5)	
	1-3	2		48 µl	
	4-6				
	7-9				
	10-12				
	13-15				
	16-17				
	18-21				
	22-24				
	25-27				
	28-30		0.5λ		
	31-34	3.64			
> dil 1/2.5	34-36	2			74°C, 10
	37-39				kill with 10µl
	40-42				0.5M EDTA
	43-45				spot 30 µl on GFC
Fidelity					
	46 ~~47~~ 47		0.5λ		
	48 ~~51~~ 49				
5 µl/pl 1-31-95	~~52-56~~				10% TCA, 1% NaPP.
dil **	50-52				5'
	53 2λ of "old mix"				↓
	54 2λ of second mix				3 x 5' in 5% TC
current 32P mix					1 x 5' in 95% ETO

pl Tag dil buffer + 5µl sample #12
5λ tag + 620λ dil buff

To Page No.____

sed & Understo d by me,	Date	Invent d by	Date
Polanco	6/9/95	Record d by	5-30-95

Project No. ______ at zero %

Book No. ______ TITLE ______ remaining (P174.9)

From I	#	Counts	in 1 ml	time	2 months	1 month
1	1	3864.00				
	2	4350.00	.024	.037	65 %	89
	3	3113.00				
2	4	155.00				
	5	130.00	0.00090	0.033	2.7%	106
	6	133.00				
3	7	4121.00				
	8	4864.00	0.029	0.032	91%	106
	9	4225.00				
4	10	3184.00				
	11	3267.00	0.021	0.029	72%	93
	12	3240.00				
5	13	3952.00				
	14	3728.00	0.025	0.033	76%	91
	15	3817.00				
6	16	2585.00				
	17	2492.00	0.017	0.027	63%	78
	18	2795.00				
7	19	4009.00				
	20	4530.00	0.028	0.033	85%	84
	21	4334.00				
8	22	4199.00				
	23	4281.00	0.028	0.031	90%	89
	24	4326.00				
9	25	4248.00				
	26	3739.00	0.026	0.031	84%	90
	27	3922.00				
10	28	65.00				
	29	86.00	0.00053	0.022	0 ~~2.4%~~	0
	30	93.00				
11	31	4340.00				
	32	3827.00	0.026	0.032	81%	78
	33	4038.00				
12	34	5155.00				
	35	5666.00	0.035	0.034	103%	—
	36	5301.00				
13	37	3927.00				
	38	3981.00	0.026	0.031	84%	97
	39	4352.00				
14	40	4214.00				
	41	3962.00	0.027	0.035	77%	83
	42	4287.00				
15	43	3992.00				
	44	3958.00	0.026	0.032	81%	106
	45	4278.00				
40°C ct	46	2014.00	0.013			
	47	2016.00				
4°C	48	1504.00	0.0098			
	49	1509.00				
Tag	50	5941.00	(.04 u/l) by definition			
	51	6122.00	6177			
	52	6468.00				
old	53	49708.00	46819			
new	54	40962.00				

same as P 36 when corrected for 30 µl on GFC instead of 40 µl and 4 day decay of ^{32}P

T Pag N

Witnessed & Understo d by me, [signature] Date 6/9/95

Invented by [signature] Record d by Dat 5-30-95

g N .

sp act

$\frac{\text{46819 cpm}}{\text{00 pmol dCTP) (4)}} = 29.3 \text{ cpm/pmol(nt) DN}$

$\left(\frac{\text{6177 avg Tag cpm}}{\text{29.3 cpm/pmol}}\right)\left(\frac{60\lambda}{30\lambda}\right) = \boxed{\text{421 pmol DNA synthesized}}$

1 unit 10 n mol / 30' at 74°C

$\left(\frac{.421 \text{ n mol}}{10 \text{ mol}}\right)\left(\frac{30'}{10'}\right) = 7.89 \text{ u/µl}$

$\frac{.04 \text{ u/µl in Rxn}}{7.89 \text{ u/µl for Tag}}\left(\frac{\text{samples cpm}}{\text{Tag cpm}}\right)$ = µl of sample

00µM each dNTP

To Page No.

ed & Understood by m ,	Date	Invented by	Date
Bolanz	6/9/95	R corded by	5-30-95

[redacted] Exhibit 99
Appl. No. 09/558,421 [redacted]

Project No.________
Book No.________ TITLE Repeat of P. 20 : stability of Tfl / Vent mixes.

From Page No.__

	A
H_2O	446.4 ✓
5X Cheng buffer (no dNTPs)	140 µl ✓
activated DNA 3.7 mg/ml	94.6 µl ✓
dA,T,G,C-TP 10 mM each	3.5 µl ✓
α^{32}P dATP 10 mCi/ml (ref 6-2-95) 3000 Ci/mmol	1.5 µl
	686

Cf=[illegible]

tube # 1-4 5-8 9-12 13-16 17-20 21-24 25-28 29-32
① ② ③ ④ ⑤ ⑥ ⑦ ⑧

A 98 µl ⟶ Vent alone ⟶

u/µl Tfl in epicentre units (its ~3.5 units really) and .09 units Vent/µl

2 λ 1x Cheng ← 0.18 unit Vent [illegible]

Tfl LTI SB 2
+Vent (5-16-95) to 7 ⟶ — (no enzyme)

Tfl Epicentre SB 2 ⟶
+Vent 5-16-95

mistake : this is 0.09 units / 100 µl . [illegible] be 0.18 - ie Vent used per 50 µl PCR * Vent [illegible]

Tfl Epicentre enzyme 2/1.8 ⟶
+Vent (5-16-95)

2 µl .045 u/λ Vent diluted in 1x Cheng buffer

2 µl of Ven[illegible] (lot #17) 2u/λ of 2-[illegible] in 86.9 µl 1x Cheng [illegible]

Vf ~ 100 µl

68°C
start with addition of enzyme to preheated mix.
remove 10 µl to 5 µl 0.2 M EDTA (spot 10 µl on GF[illegible]
and 5 µl to 5 µl Killing solution with cold dAM[illegible]
(spot 2λ) on PEI at 0, 5, 10, 15, 20 min.

resolve in 1M LiCl

T Pag N

Witnessed & Understood by me, [signature] | Date 6/9/95 | Invented by [signature] Recorded by | Date 5-31-95

N

cpm − background cpm / specific activity = pmoles

pmoles / ave pmoles incorporated = % turnover — should be constant; 2 replicates of each time pt.

		CPM1	pmoles	% turnover
1	5'	885.00	ex 885−543 / 103.31 = 3.31	0.042
2	10'	1241.00	4.76	0.069
3	15'	1074.00	5.14	0.065
4	20'	1269.00	7.03	0.062
5		984.00	4.27	
6		1332.00	7.64	
7		1678.00	11.0	
8		1590.00	10.1	
9		830.00	2.78	
10		1213.00	4.49	0.021
11		1195.00	6.31	0.066
12		1460.00	8.88	0.055
13		555.00	0.116	0.062
14		1228.00	6.63	
15		1225.00	6.60	
16		1425.00	8.54	
17		764.00	2.14	
18		977.00	4.20	
		CPM3		
19		1212.00	6.48	0.039
20		1453.00	8.51	0.028
21		895.00	3.41	0.043
22		772.00	2.22	0.061
23		1009.00	4.51	
24		1365.00	7.96	
25		746.00	1.96	0.70
26		438.00	−1.82 X	0.47
27		757.00	2.07	0.14
28		609.00	0.04	
29		412.00		
30		578.00	x̄ 543	
31		488.00	background,	
32		693.00	no enzyme	

} 1 replicate/time pt

result: 1) turnover began to reach a plateau by 10 min. We expected turnover to continue increasing over time after DNA synthesis stopped
- Tfl completes w/ Vent at nick

2) turnover by Vent alone in Cheng buffer is lower than expected. 25% turnover was observed in another experiment
453 pmole in 20 min / 25 = 1/25 syn by vent
The high signal to noise level for the Vent samples makes it difficult to say what the turnover is. Turnover is higher when Vent is mixed w/ Tfl.
- Tfl creates mismatches that are targets for Vent exo

do: repeat w/ 1, 2, 3, 4, 5 min time points and more Vent enzyme in the Vent alone samples, use new PEI plates

specific activity: cpm of 2ul spot of mix A (2 replicas) 36438, + 46210 — x̄ = 41,324 cpm

$$\frac{\left(\frac{100\text{ul rxn}}{2\text{ul spot}}\right)\left(41324\text{ cpm}\right)}{\left(5000\ \frac{\text{pmole}}{\text{nt}}\right)\left(4\text{ bases}\right)} = 103.31\ \frac{\text{cpm}}{\text{pmole (nt) DNA}}$$

50uM each dNTP in 100ul rxn
50 umole/L x 100 x 10⁻⁶ L = 0.005 umole = 5 nmole = 5000 pmole

To Pag No.

I & Understo d by me,	Date	Inv nted by	Date
Polanyi	6/9/93	Recorded by Carolyn Combs	5-16-95

From Page No.__

$\frac{\text{sample cpm}}{\text{specific activity}} \times \frac{100\,\mu l\ rxn}{10\,\mu l\ spot} \times \frac{15}{10}$ dilution

		Incorporation	pmoles
1	1	49596.00	49596 / 103.3 × 15 = 7,201
	2	74066.00	10,754
	3	88521.00	12,853
	4	95661.00	13,889
2	5	50395.00	7,317
	6	69543.00	10,097
	7	82738.00	12,013
	8	93515.00	13,578
3	9	45114.00	6,550
	10	64768.00	9,404
	11	81250.00	11,797
	12	96711.00	14,042
4	13	49095.00	7,128
	14	71796.00	10,424
	15	81335.00	11,809
	16	95798.00	13,909
5	17	50290.00	7,302
	18	70938.00	10,230
	19	88754.00	12,887
	20	98147.00	14,250
6	21	48881.00	7,097
	22	85245.00	12,377
	23	85694.00	12,442
	24	91420.00	13,274
7	25	1932.00	281
	26	2581.00	375
	27	3000.00	436
	28	3120.00	453
8	29	854.00	123
	30	777.00	113
	31	183.00	26.6 (no enzyme)
	32	487.00	70.7 (no enzyme)
		~~9.00~~	
		~~6.00~~	
2ul mix A =		36438.00	$\bar{x}$ 41,324 cpm
		46210.00	

for calculation of specific activity

103 cpm/pmol

Synthesis ~~first~~ was almost complete by 10min
By 20min ~14nmoles of the 20nmole had been incorporated - hi, in the 1st

To Page N

Witnessed & Understood by me,	Date	Invented by	Date
DPolanco	6/9/95	Recorded by	5-31-95

new dilution of r log lot EKBT1
to 5u/µl.

Proj ct N .______
Bo k N .______

ge N .__

EKBT1 157.2 µl
323 u/µl (see p91, 9)

Tog storage buffer 10 ml
(Pierce detergent) Vf = 10.157 ml (Cf = 5 u/λ)

mix end over end 1 hour

storage buffer is from 12-7-94
with Pierce detergents

To Pag No.____

ed & Understood by me, Polanyo | Date 6/9/95 | Invented by [signature] | Dat 5-31-95
Recorded by

Exhibit 101
Appl. No. 09/558,421

Project N

44 Book No.____ TITLE primer degradation using Tfl (can see P 17, the section #11)

From Pag No.__

	(1)	(2)	(3)	(4)	(5)	(6)
5 X Cherry (no dNTP's)	20 µl →					
32P "33 mer correct" same as (P12 and 14) 5 µM	4 →					
Tfl/Vent 5-16-95 CTI 5B	10					
Tfl/Vent 5-16-95 Epicentre 5B		10				
Tfl/Vent 5-16-95 Epicentre Tfl			10			
Tfl LTI 4.33 u/µl (P.8)				10		
Vent .09 u/µl lot #17 (opened 2-24-95) LTI 5B P.6 (same stock as in Tfl P.8)				10		
H2O	66 →					
Vf = 100 µl						

(0.9 units va in 100 µl re

* 2 u/µl Vent diluted into CTI Taq 5B
2 µl Vent
42.4 µl Taq 5B
44.4 Vf

68°C. remove 10 µl to 5 µl agile rxn
stop rxn at 0, 5, 10, 20, 40, 60, 80, 100 m

● add enzyme on ice → take 0 time point, start timing when thin walled tube put in preheated 960° at 68°C

16 hrs PAGE ~44 watts (Volts range from 160
get ~ 12 cm/hr for BPB

T Page N

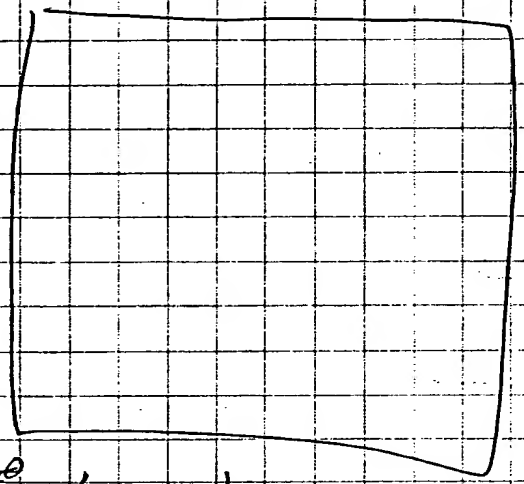
Witness d & Und rstood by me,	Date	Inv nt d by	Dat
[signature]	6/9/95	R c rded by	6-2-95

ag N .__

32P-33mer is ~ 10 x 10^6 CPM/µl
↓ dilute 1/1000

1.5 x 10^-6 M
5' round nt of 23mer ~ 20 mM nt
5µl H2O
Fill

10mM dATP stock



comm stock
1:5 dilution
1 out stock, 4 out H2O TE

M ATP
M dATP

M 23mer
ved

-3mer
1/1000

To Pag No.____

sed & Understood by me,	Date 6/9/95	Inv nted by	Date 6-2-95
Doolan		R cord d by	

[redacted]

Project No.________

46 Book No.________ TITLE ____________________

From Page No.__ Results:

1. ice does not shut down 3' exo (start with Mg repl (?))

2. there is very rapid loss of first 1-10 nts, then very slow degradation i.e. with time, need less enzyme and/or shorter time points

3. No apparent endo or 5' exo activity for Tfl alone as seen on P14. Here its 5λ Tfl (4.33 u/λ) per 50 μl compared to 2λ on P14 but still should see plenty of loss of full length here based on rate seen on P14. (also was 1X buffer on P14) Try ± dNTPs
also P14 has dNTPs present but not here. Maybe be some kind of primer extension involved in loss of full length primer — ie extension to many lengths contributes to apparent loss of primer or to production of a 5' exo target — could be primer dimer extension or hairpin within 33 mer.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/9/95	[signature]	6-2-[illegible]
		Recorded by	

g N .__

95 - 6/6/95

pose: To measure 3'→5' exonuclease activity of TFI/Vent using the primer degradation assay.

kground: An earlier trial of this experiment (NB 10 page) was done by removing aliquots of the rxn at time points 0, 5, 10, 20, 40, 60, 80, 100 min. The primer was degraded almost to the maximum amount by 10 min. Since we want do determine the 3'-5' exo activity rate, we need to find the linear range of the assay. This can be done by taking shorter time points or by taking a single time point on a series of enzyme dilutions (doubling [enzyme] should double extent of degradation in the linear range of the assay) We'll do this trial expt w/ just 1 enzyme sample - TFI/Vent in LTI SB - and 13 different dilutions. Once the linear range is found, we can repeat the exp. just on that range.

terials: ^{32}P γATP for end labeling primer
primer = 33mer correct
Taq dilution buffer - cc aliquot
LTI storage buffer - RL aliquot
TFI/Vent enzyme mix - from stability study
9600 PCR machine + tubes
5x Cheng buffer - cc aliquot
PNK = T4 kinase + buffer - ~~old~~ - LTI new
8% sequencing gel + buffer - LTI premade
Stop buffer
sterile H_2O

To Page No.____

d & Understood by me,	Date	Invent d by	Date
Polaroid	6/9/95	Recorded by Carolyn Coombs	6/9/95

Project No. _____

2 Book No. _____ TITLE _____

From Page No. ___

Procedure

6/5 ① end-label the 33-mer primer w/ ^{32}P γ ATP = Kinase reaction

reference = notebook 10 p 12

mix:	H_2O sterile	———	25ul ✓	+ 25ul
	5x Kinase buffer		12ul ✓	+ 12ul
20uM	33mer correct constant,	p 133 20 pmole/ul	15ul ✓	+ 15ul
AC4521	^{32}P γ ATP 10mCi/ml	ref	5ul ✓	+ 5ul
	PNK 10u/ul	new FE5419	3ul	+ 3ul
			60ul	/ 120ul

5 added after 1st 5 co were added

incubate 37°C, 30min ✓

~55°C, 5min ✓

store labeled DNA + unincorporated label at -20°C — run some ol

6/5 ② make a ~~8~~ 8% denaturing-sequencing gel + buffer → both premade by

- 1,75ml bottle of 8% mix (cold room) + 450ul APAP (made fresh)

  0.0868g AP ←

  368ul H_2O ⇒ $\frac{.0868g}{.368ml}$

- after pouring gel, shake up remaining gel mix so it can be used to fill leaks, ect.

- store gel upright, ON, at RT w/ H_2O-soaked towels and saran ✓

T Pag N

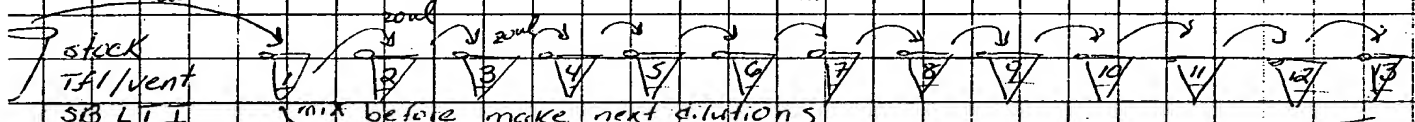
Witn ss d & Underst d by m ,	Dat	Invent d by-	Dat
	6/9/95		6/9/95
		R c rded by	

Pg N .__

et up enzyme reactions ; ~~44 rxns~~ for 16 rxns:

Master mix A = ~~87 ul~~ 89.6ul H_2O ✓
per rxn → 5.4ul H_2O ~~3 ul~~ 6.4ul ^{32}P 33-mer ✓
0.4ul ^{32}P 33-mer 30 ul 5X Cheng buffer 32ul, 5x cheng ✓
2 ul 5x Cheng ~~120ul~~ 128ul
8ul enough for 15 rxns
store on ice

enzyme dilutions: TFI/Vent in SB LTI diluted in Taq dilution buffer
- 20ul Taq dilution buffer in tubes 1-13, big tubes
- add 20ul TFI/Vent to tube #1 = 1:2
- ~~vortex~~ flick to mix and store on ice
- remove 20ul from tube #1 into tube #2 = 1:4 ect



mix before make next dilutions
each w/ 20ul Taq dilution buffer

- store dilutions on ice

add 8ul MixA to 9600 tubes # 1-14 * radioactive and prewarm to 68°C in 9600 machine

Tube #	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mix A 8ul	equilibrated to 68°C →													
Dil TFI/Vent 2ul														
Dilution factor = SB LTI	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/250	1/512	1/1024	1/2048	1/4096	1/8192	no enzyme 8ul Taq dil buffer

add enz. to tube, flick to mix, stagger rxns by 1 min

After 15min, remove tube and add 5ul stop - pipet up + down 3x and keep on ice until gel is run

gel, run ~~15, 1-15~~ ~~14, 1-13, 14~~ (3ul) ~~old labeled 32P-labeled 33-mer from 6/1/95~~ 3ul old + 12ul H_2O remove 2ul + 2ul 5x cheng + 6ul H_2O w/ more label

— load 2ul old + 10ul H_2O 2ul 5x cheng 4ul H_2O 3ul d.i. load 3ul 2ul + 548ul H_2O 3ul 5ul stop

at before loading, 95°C 5min in 9600
in down 11:20am 1700V ⇒ 65W

To Page No._____

Read & Understood by me, [signature] Date 6/9/95

Invented by [signature] 6/9/95 Date 6/9/95

Recorded by [signature]

Project No._____

Book No._____ TITLE _____

From Page No._

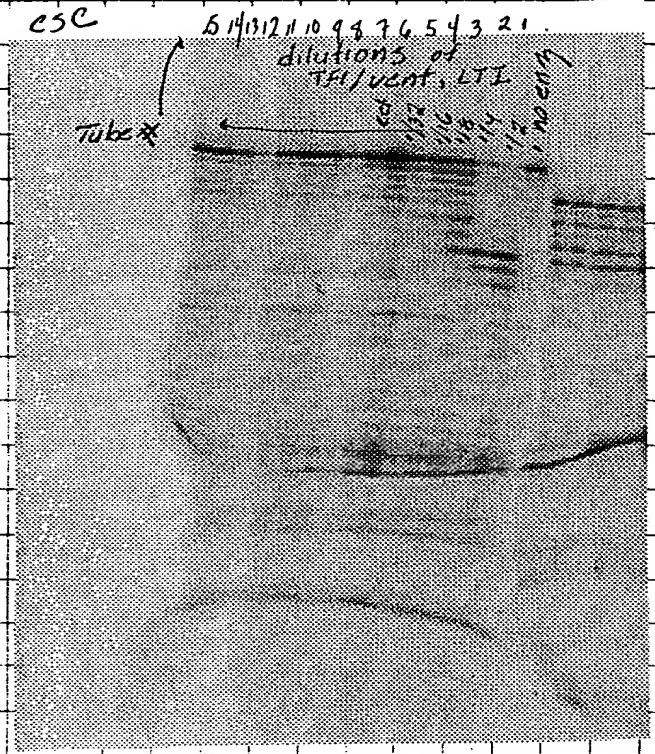
- B&B ran down to bottom glass clamp on gel rig, ~1hr, 1700 V (constant), ~65W
- transfer to drain buffer Whatman paper,
- cover w/ saran & cut to size
- dry - 2 pieces whatman under gel, saran over gel, dry ice in trap
- set vacuum & heat for 1hr = 11 - 12:45 - 1:45 PM
- set in phosphoimager cassette - bottom to bottom w/ saran ~2 PM for ON exposure

Result: ON exposure on phosphoimager

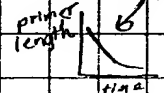
csc

C:\DATA\CC.GEL 1995:06:07 07:57:48, Range = 0.11-10000.00 Counts, 0.50x

csc



Conclusion: The 1/4, 1/8, 1/16 dilu
~~gave~~ span the linear range
the primer degradation
Now, we'll do a co
time pts of each
dilution to gather
better data from the lin
range The data w/
used to show 3-5 exo ac
for stability study



T Pag No

Witn ssed & Understood by m, D Polan

Dat 6/9/95

Inv nt d by 6-9-95

Rec rded by Carlos Combs

Dat 6/9/95

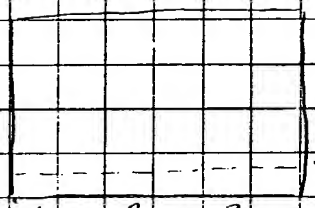
Page N ... 6/6/95

Purpose: To determine the relative mobility of the ^{32}P-33mer correct primer on a PEI plate, developed in LiCl.

Background: Originally we wanted to determine the specific activity of the ^{32}P-33mer primer that was used in the primer degradation assay w/ TFI/Vent (NB 10 p) However, we later decided that it is not important to find the specific activity since we can do a no enz. control each time the assay is done. Now we want to determine the mobility because we observed that the cold oligo did not run as expected on the PEI plate, and we just are curious about ~~how~~ where the oligo ran.

Materials: cold 33-mer correct
^{32}P 33mer correct - labeled on 6/9/95
ATP
PEI plate
1M LiCl
scint vials + cocktail

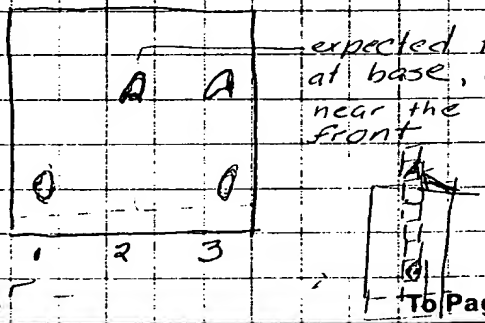
Procedure: > spot on PEI plate →



	1	2	3
20mM ATP	2ul		2ul
20mM 33mer		2ul	2ul
1/1000 ^{32}P-33mer			2ul

> set plate in developing chamber w/ 1M LiCl ~1hr - ran 1/2 way
> circle control spots (ATP + cold 33mer) in lane 3 under UV light → sketch of how plate looked

{ γATP^{32}*
ADP
^{32}P-33mer*
cold 33mer
PP$_i$ ^{32}* }



> cut lane 3 into 8 pieces and count in scintillation counter -

To Page No.

Read & Understood by me,	Date	Invented by	Date
Polanyo	6/9/95	6-12-95	6/9/95
		Recorded by Carolyn Evans	

From Page No.__

C.5.C

Result:

PAGE:

USER: 1 ID:32P 1.0 CPM PRESET TIME: 1.00 TUE 06 JUN 1995 15:36
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
H#: 0 ADC:N QCF:N RCM:N
CHANNEL 1-LL: 0 UL:1000 2SIGMA: 0.05 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR:0 1.00000
HALF LIFE(DAYS):N

SAM	CPM1		TIME	ER
1	111.00	- baseline	1.00	
2	307.00	- area around where cold ATP standard ran	1.00	
3	136.00		1.00	
4	117.00		1.00	
5	60.00		1.00	⇒ primer runs like dAMP
6	215.00	- area around where cold 33mer primer ran	1.00	
7	27.00	- near solvent front	1.00	

The labeled 33-mer primer ran like the dAMP runs on a PEI plate, up near the solvent front, not at the origen.

This result was expected from the information we heard from the chemistry group: DNA stays at the origen because of its large size.

oligonucleotides run like dNMP because they have same charge:mass ratio as dNMP

To Pag N

Witness d & Und rsto d by m , [signature] Dat 6/9/95

Invent d by [signature] 6-12-95 Dat 6/9/95

R c rded by Pauline Pond

Tfl/Vent primer degradation assay - time course on serial dilutions for a time point of stability study

Page No.

Purpose: To measure 3'-5' exonuclease activity of Tfl/Vent mixes in 3 buffers at the zero time point of stability study

Background: Linear range of assay determined in previous expt, NB 11 page 1 - dilutions 1/4, 1/8, 1/16 looked good
- linear range ↳ = 0.045 units
- now do time course of these dilutions

1/4 dilution → $\frac{0.09\ u/ul}{4} \times 2ul = 0.045$ units in 10ul reactions 1/8 dil ⇒ 0.0225 units 1/16 ⇒ 0.0113 u

Materials: ^{32}P 33mer correct - labeled on 6/5/95
Tfl/Vent in LTI, epicenter SB, epicenter Tfl
8% gel
mix A

Vent dilution 2000 u/mL
2ul Vent Lot 17 2/24/95
42.4ul Tag 3/3
44.4ul at 0.09 u/ul
22.2x dil

Procedure:
1) make mix A, enough for 15 (14) rxns - 90ul per rxn

	per rxn	15 (14) rxns
Tfl/Vent in LTI SB	20ul	300 (280)ul 5x Cheng
" in epicenter SB	4ul	60 (56)ul ^{32}P 33mer 5um stock
" in epicenter Tfl	66ul	990 (924)ul H_2O
Vent	90	1350 (1260)ul mix A

1 no enz

2) each enzyme/buffer mix

	stop tubes #'s					
		37-40	41-44	45-48		
		25-28	29-32	33-36		
		13-16	17-20	21-24		
		1-4	5-8	9-12		
mix A		90ul	90ul	90ul		
H_2O		8	6	4		
undiluted enzyme (0.09 u/ul)		2	4	6		

0-15 12
14 11
13 10

take 3, 6, 9, 12 min time points by removing 10ul of rxn to 5ul stop in small tubes, keep on ice

start rxns 1 min apart time on clock - 0 start, 1, 2, 3 stop, 4, 5, 6 stop, 7, 8, 9 stop, 10, 11, 12 stop, 13, 14 stop

1, no enzyme control (5ul mix A, 5ul H_2O, 10ul Cheng, 2ul 32P 33mer, 85ul H_2O → 5ul + 2.5ul stop / 50ul)

To Page No.

Read & Understood by me,	Date	Invented by	Date
Polanco	6/9/95	6-12-95	6/19/95
		Recorded by	
		Cindy Lomax	

Project No._____

10 Book No._____ TITLE _____

From Page No.__

Note: samples Vent / TfI in LTI, 3mm 2ul, 3mm 4ul > only 5ul of can was stopped - load mas 4.5 inste

4:50 PM - ~6:15 PM 1700 V constant, ~45 W, gel was dried & put in P.I

* 39 & 46 may be underloaded due to problem expelling full vol. from

order: control, 1 → 48 where 1-12 are TfI/Vent in LTI SB

of samples on gel 13-24 are TfN/Vent in epicenter SB

25-36 are TfI/Vent in epicenter TfI

37-48 are Vent alone

T Pag N

Witn ss d & Und rsto d by m , D Polanz

Dat 6/9/95

Invent d by 6.12

R cord d by Carolyn Coombs

Date 6/9/95

Exhibit 105
Appl. No. 09/558,421

Project No.

48 Book No. TITLE Repeat unit assay for Tf1/V. incorp of P.30 for stability of Virus Tf1/Vent mixes of #'s J-5 with 5 duplicate dilutions

From Page No.

Use 2 µl/of 1/250 dil for optimized signal/noise and linearity vs (units)?

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Tf1 Chem unit assaying (same as P. 30) 47 →

Tf1 4.33 u/µl (P.T) 1/250 dil * 2 →

Tf1/Vent LTI SB S-16-97 (Ning Guan's notebook) 1/250 2 →

Tf1/Vent Epicenter SB S-16-55 1/250 dil 2 →

Tf1/Vent (Epicenter Tf1) S-16-55 1/250 dil 2 →

Vf-50µl → 14°C, 10' → 10µl 0.5m EDTA → spot 40λ on

* all dilutions are done as 5 separate dilutions of 2µl Enzyme into 498 µl Tag dilution buffer

T Pag

Witness d & Understood by me, Dat 6/9/95 Inv nted by Dat 6-9-95

R c rded by

ge N .__

1	4163.00
2	4429.00
3	4636.00
4	4646.00
5	4349.00
6	4550.00
7	4529.00
8	4623.00
9	4350.00
10	4315.00
11	3995.00
12	4339.00
13	3732.00
14	4695.00
15	4428.00
16	3975.00
17	4584.00
18	4541.00
19	4297.00
20	4412.00
21	259.00
22	84613.00
23	87557.00

T Page No.____

sed & Und rstood by me,	Date	Inv nted by	Date
	6/19/95	Recorded by	6-9-95

Test run of PEI plates - prior to turnover expt

No.

1/95

pose: To test how well a fresher batch of PEI plates can resolve dAMP from dATP and how tight/clean the spots are. This is being done prior to using this batch of plates for another TFI/Vent turnover experiment

ckground: Last time the dAMP spot did not resolve well from the yellow "junk" that runs near the 1M LiCl solvent front, making it difficult to cut out + count just the dAMP (without Pi) for accurate turnover results. We'll try washing a different batch of plates - from Jesse - in dH_2O + drying them 1st, before running samples and compare to unwashed plate. Also we see if running a whole plate gives better resolution than a ¼ plate

terials: PEI plates - from Jesse ← Machery Nagel Polygram cell 30 PEI/UV
Aldrich cat # 12,288-2
Kill soln = ~20mM dATP
20mM dADP
20mM dAMP
100mM CDTA
1M LiCl - Fresh, see recipe on next page

To Page No.____

d & Understood by me, | Date | Inv nted by [signature] 6/19/95 | Recorded by Carolyn Coombs | Dat 6/12/95

see P14 apparent exo present in PCR buffer

Project No.

Book No.

TITLE Repeat apparent exo result for Tfl on P14 with different primer

50 P44, no exo in Chemo buffer

From Page No.

Exhibit L-107
Appl. No. 09/558,421

	①	②	③	④	⑤	⑥	⑦	⑧	
10X PCR buffer	5 →						5		✓
50 mM $MgCl_2$	to 5μl →						15		✓
5X Chemo (no dNTPs) (see P200)				10μl →			10		✓
32P 33mer correct 5μM (PJ1)	2			2					✓
32P 23mer "AC" 5μM		2			2				✓
32P 42mer "fidel" 5μM			2			2			✓
see P12, 14 for method									
+Taq storage buffer							10	10	
Tfl 4.33u/μl (P.8)	X10 →								
H_2O	31.5 →			28 →			31.5	28	

V_f = 50μl

74°C. remove 10μl to 5μl cycle seq stop sol at 15 min, 60 min

run on 8% PAGE

* Zero time point:

1. mix buffer^{mix}, ^{32}P primer (MgCl2 if needed) and H_2O. Volume =
2. remove 8μl to 2μl Taq storage buffer and 5μl cycle seq stop for 0 (no enzyme) time point.
3. now have 32μl of reaction^{mix} left. preheat to 74°C, add 8μl Tfl so V_f = 40μl again and remove 10 at 15 and 60 min to 5μl cycle seq stop sol.

To Pag

Witnessed & Understood by me,	Date	Invented by	Dat
Deanna Bolan	6/1996	Recorded by	6-15-97

32P oligos (follow p12, 14 for 5μM Cf.

Project No.

Book N

ag N

	1	2	3	
5X Kinase buffer	4 μl	→	→	✓
"arrest" 20 μM 1389	5 μl			✓
mer AC (P136,5) 100 μM		1 μl		✓
mer "fidel" 6-13-95 "old Temp." This is different from Fidel Temp P54 100 μM			1 μl	✓
ATP 1 μCi/μl (ref 6-16-95)	2 μl	→	→	✓
VR	1 μl	→	→	
H2O	8	12	12	✓
	F = 20 μl			

← 23mer has 3' terminal A instead of G its called "AC".

← (5X less γATP than on p12)

37°C, 30', 5', 55°C

22 mer ~~fidel~~ fidel

51351 CR (012)

56.89 nmoles primer
568.9 μl H2O

F = 100 μM

To Page No.

Read & Understood by me, [signature] Date 6/19/95

Invented by [signature] Date 6-13-95

Recorded by

Incorporation and turnover by Vent alone
Time course on 0.1, 0.2 0.3 units Vent

Page No.

Purpose: To measure both incorporation and turnover by varying amounts of Vent over a 1 hr time course. These values will be compared to those of TFI/Vent mixes. Vent alone is a control for the stability assays of TFI/Vent mixes.

Background: NB 10 p. 41, turnover was not above background for Vent alone, when 0.09 units were used in a 20min time course. This time will try more enz. However, Roger had observed turnover over background when 0.15 units were used /100ul rxn. Turnover is expected to increase linearly w/ time even after incorporation has stopped, if Vent can bind at the nick. New PET plates may give better results than last time.

Materials: PET plates from Jesse, tested on NB 11, p. 15, Vent 2u/ul, Kill soln = NB 11 p. 14 → 20mM dATP, dGTP, 100mM EDTA
activated DNA = gapped DNA made w/ DNase I
^{32}P αdATP - ref date 6/16/95

mix A: per 1, 100ul rxn:
- 63.786 ul H_2O
- 20ul 5x Cheng buffer
- 13.5ul activated DNA, 3.7 mg/mL, Cf = 0.5
- 0.5ul dATGC-TP, 10mM each, Cf = 50u
- 0.214 ul α ^{32}P dATP 10mCi/ml

make enough mix A for today + the next expt - TFI/Vent turnover
16x 98ul/rxn = 1568ul

(For 16 reactions) A
- 1020.6ul H_2O go 1mL + 20.6ul ✓
- 320ul 5x Cheng ✓
- 216ul DNA ✓
- 8ul dATGC-TP - BRL Lot FB14001 ✓
- 3,424ul ^{32}P αdATP

7.5 x 10^7 cpm/ul
~~4.7 x 10^8~~ ~~294~~ 250 / 100 µl Reaction
7 x 10^6
VF 1569 µl use 98 µl / 100 µl Rxn

Vent dilutions - 2/4/95 Lot 17 spin + mix of Vent
LTISB = (1aq 5B)
~~1st dilute 5x Cheng → 10ul 5x Cheng + 40ul H_2O - mix~~
① dilute Vent stock (2u/ul) to 0.15u/ul → 2ul Vent + 24.66ul LTISB Cheng
② dilute ① to 0.1u/ul → 10ul ① + 5ul ~~1x Cheng~~ LTISB
③ dilute ② to 0.05u/ul → 5ul ② + 5ul ~~1x Cheng~~ LTISB

Page No.

Read & Understood by me,	Date	Invented by	Date
Lucia Polcyn	6/19/95	6-19-95	6/13/95
		Recorded by Carolyn Combs	

From Page No.

procedure: - deliver 10ul of kill soln to 1.5mL Eppendorfs x 4
- label tops 10' 20' 30' 40' 50' 60', set on ice until

rxn #	1	2	3	4
stop tube #	1 - ~~16~~	~~7~~ - ~~12~~	~~13~~ - ~~18~~	~~19~~ - ~~24~~

mix A 98ul/rxn — prewarm to 68°C →

0.15 w/ul Vent 2ul start rxns by adding enz., keep at 68°C in 9600

Lot# 17
opened 2-24-95

2ul ← Thin in [illegible] TF1/Vent

0.10 w/ul Vent

0.05 w/ul Vent 2ul

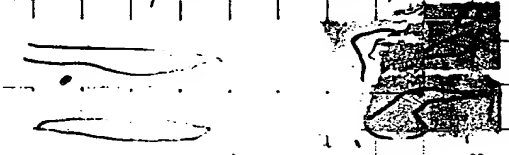
0.2 ul [illegible] TF1 [illegible]

no enz 2ul ~~tr Cheng~~
LTI $B
= Taq $B

100ul

- remove 10ul rxn to stop tubes at each time pt (10', 20', 40', 50',
- spot 10ul/GFC + 2ul PEI
L+ 10ul of mix A x 3 (for determination of specific activity)

Test of old (Baker) PEI plates - 2ul, cold kill soln spotted



- old plate → dAMP runs w/ solvent front junk

EC

- new plate → dAMP runs ~½ between solvent front & or

T Pag N

Witness d & Und rst od by me:	Date	Invent d by	Dat
Deanna Polump	6/19/95	6-15-95	6/13/95
		R cord d by Carolyn Combs	

Exhibit **109**
Appl. No. 09/558,421

Project No._____

Book No._____ TITLE Incorporation (pmoles)

From Page No.___

$\left(\frac{cpm}{\text{specific activity}}\right)\left(\frac{100\,ul\,rxn}{10\,ul\,spot}\right)\left(\frac{20\,ul}{1\,ul}\right)$

3 mins	55	21752.00 - 936 pmoles
	56	40494.00 - 1742
	57	50701.00 - ~~2745~~ 2181
	58	63810.00 - ~~2723~~ 2745
	59	63423.00 - 2723
	60	61923.00 - 2663
1 min	61	12710.00 - 547
	62	21727.00 - 934
	63	32040.00 - 1378
	64	39939.00 - 1718
	65	43064.00 - 1852
	66	51401.00 - 2211
0.1 mins	67	9060.00 - 390
	68	14810.00 - 637
	69	19948.00 - 858
	70	24421.00 - 1050
	71	31940.00 - 1374
	72	30490.00 - 1311
no enz.	73	420.00
	74	540.00
	75	299.00 $\bar{x} = 348$
	76	310.00 $n = 6$
	77	197.00
	78	323.00
	79	923,719.00
	80	973,931.00
	81	890,737.00

10ul spot of mix A for specific activity

$\bar{x} = 929,402$

The observed specific activity of mix A is 2x higher than anticipated by the following calculation:

$$\frac{10\,\frac{uCi}{ul}}{0.925^{2\,days\,to\,ref.\,date}} \times 2.2\times10^{6}\,\frac{cpm}{uCi} = 2.6\times10^{7}\,\frac{cpm}{ul} \times 3.4\,ul\,in\,mix\,A = \frac{8.7\times10^{7}\,cpm}{1568\,ul\,A} = 5.6\times10^{4}\,\frac{cpm}{ul}$$

$5.6\times10^{4}\,\frac{cpm}{ul\,A} \times 10\,ul\,A\,spotted = 5.6\times10^{5}\,cpm\,expected$

$9\times10^{6}\,cpm$

$9.3\times10^{5}\,cpm\,observed$

I don't know where the error came from, but the results should still be consistent within this experiment

To Pag No

Witnessed & Understood by me, Dat 6/19/95

Invented by 6-19-95 Dat 6/14/95

Recorded by

Tfl/Vent in LTI SB: turnover and incorporation 5 replicates of 3 time points

ge N .

pose: To more accurately determine turnover by Tfl/Vent in LTI SB
- 5 replicates of the 5min and 10min +15min time points, ~~which~~ are within linear range of the assay. This data will used for the stability study.

ckground: > also see NB 10 page for a time course of turnover, 2 replicates

aterials: new PEI plates,
mix A from 6/13/95
Tfl/Vent in LTI SB
Kill soln from NB 11 page 14 = 20mM each dA-mDTP-P
100mM EDTA

ocedure: - deliver 20ul Kill soln to 1.5ml stop tubes 1–18

rxn # s (4 tubes)	1	2	3	4	5	6
top tube #	1-3	4-6	7-9	10-12	13-15	16-18

mix A 98ul → prewarm to 68°C

wick plate

l/Vent mix 2ul - add enzyme to start rxns
TI SB 2ul
2ul
2ul
2ul
2ul
~~2ul~~ ~~no enzyme control~~
2ul LTI SB = Taq SB

incubate at 68°C
100ul
> At 5, 10, 15 min remove 20ul rxn to the 20ul Kill soln in stop tubes, mix well & keep on ice
> spot 10ul/GEC filter (1-15 / +A 16-18) 3 + 12 = 27 spot rxn 6, no enz control 4X per time pt
2ul/PEI plate (1-15) 27 x 2 = 54 = 12 spots
16-1, -2, -3, -4
17-1, -2, -3, -4 18-1, -2, -3, -4 =

To Page No.

d & Understo d by me,	Dat	Invented by	Date
[signature]	6/19/95	[signature] 6-19-95	6/14/95
		Recorded by [signature]	

[redacted]

Project No.

22 *Results:*

Book No. TITLE

Turnover (pmol)

$(cpm - background\ cpm / specific\ activity^{*})(\frac{40}{20})(\frac{100}{2})$

SAM (odd = dAMP, even = dADP or dATP)	CPM1	Turnover (pmol)
1 > 5	950.00	$(950-495/419)(\frac{40}{20})(\frac{100}{2}) = 109$
2	72035.00	
3 >	1727.00	~~163~~ 294
4	71656.00	
5 > 15′	2488.00	476
6	67554.00	
7 > 5′	1176.00	163
8	72213.00	
9 > 10′	1768.00	304
10	69650.00	
11 > 15	2189.00	404
12	69544.00	
13 > 5′	1219.00	173
14	71519.00	
15 > 10′	1784.00	308
16	70384.00	
17 > 15	2314.00	434
18	69324.00	
19	1189.00	166
20	71061.00	
21	1914.00	339
22	72483.00	
23	2096.00	382
24	67757.00	
25	1032.00	128
26	70489.00	
27	1714.00	291
28	64939.00	
29	2289.00	428
30	64174.00	
31	612.00	
32	75976.00	
33	494.00	
34	79452.00	
35	421.00	
36	79068.00	
37	587.00	
38	78840.00	
39	460.00	
40	80087.00	
41	481.00	
42	78697.00	
43	419.00	
44	78893.00	
45	404.00	
46	76101.00	
47	450.00	
48	68672.00	
49	549.00	
50	67776.00	
51	498.00	
52	73381.00	
53	559.00	
54	72680.00	
55	620.00 spot near dAMP	
56	1714.00 spot near dADP/TP	

(Margin labels: samples 1–6 "T51/vent replicate 1"; 7–12 "replicate 2"; 13–18 "replicate 3"; 19–24 "replicate 4"; 25–30 "replicate 5"; 31–38 "4 spots no enz"; 39–46 "4 spots no enz"; 47–54 "4 spots no enz")

$\bar{X}$ Turnover ± 1SD (n = 5)	% Turnover 100x (pmole dAMP / incorpora[ted])
5′ ⇒ 148 ± 28	3.3%
10′ ⇒ 307 ± 19	5.0%
15′ ⇒ 425 ± 35	5.5%

background for turnover
$\bar{x}$ = 495 ± 69 cpm = 118 pmoles → samples are only 1 to 3 above background
n = 12

ee 6/14/95

T Pag N

Witnessed & Understood by me, [signature] Date 6/19/95

Invented by [signature] 6-15-95 Date 6/14/95

Recorded by [signature]

Page No.

Incorporation (pmol)

(cpm / specific activity)(100ul rxn / 10ul spot)(40ul / 20ul)

5'	93072.00	93072/419)(100/10)(40/20) = 4443
10'	107957.00	- 5153
15'	140583.00	- 6710
5'	107888.00	- 5150
10'	116159.00	- 5545
15'	157153.00	- 7501
5'	89224.00	- 4259
10'	129878.00	- 6199
15'	158185.00	- 7551
5'	86678.00	- 4137
10'	129770.00	- 6194
15'	146342.00	- 6985
5'	71757.00	- 3425
10'	127388.00	- 6081
15'	158825.00	- 7581

$\bar{X} \pm 1SD$ Incorporation

$n = 5$

5' ⇒ 4283 ± 620 pmol

10' ⇒ 5834 ± 467 pmol

15' ⇒ 7266 ± 395 pmol

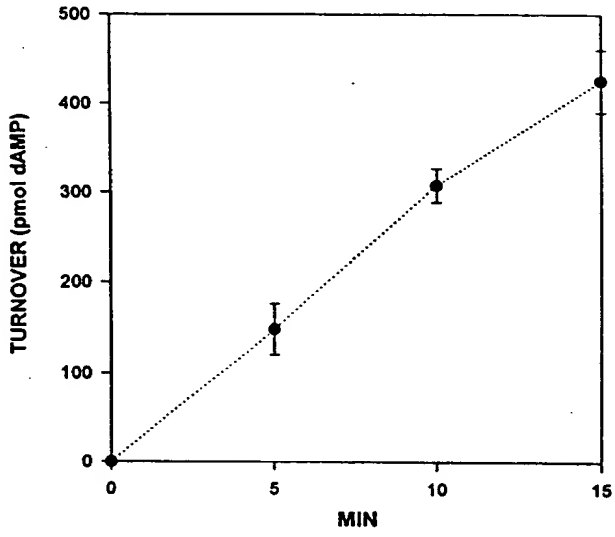
no enz 5'	285.00	background
	355.00	$\bar{X}$ = 301 ± 31 cpm
no enz 10'	291.00	= 14.4 pmoles
	300.00	n = 4
no enz 15'	310.00	
	262.00	
	839570.00	10ul mix A
	831885.00	spotted 3X
	840299.00	$\bar{X}$ = 837,251

Specific activity = $\frac{837251 \text{ cpm} \times \frac{100\text{ul rxn}}{10\text{ul spot}}}{(5000 \text{ pmol})\,4} = 419 \frac{\text{cpm}}{\text{pmole (nt)}}$

To Page No.

Witnessed & Understood by me, Barbara Polanyi	Date 6/19/95	Invented by [signature] 6-19-95	Date 6/14/95
		Recorded by Carolyn Combs	

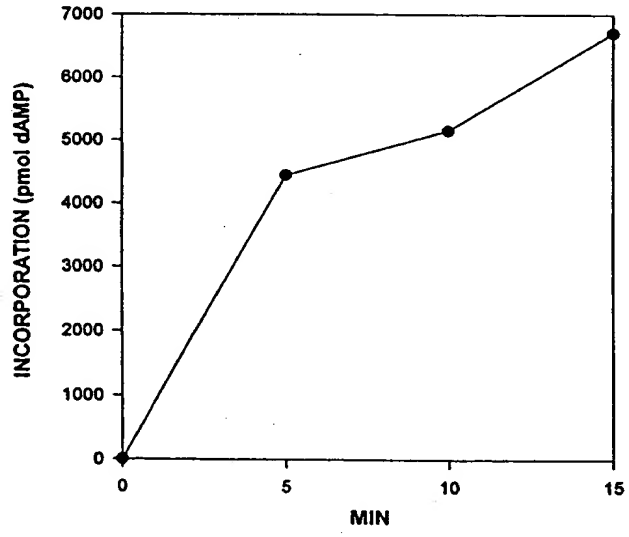
PROOFREADING: Tfl/VENT



6/15/9
ec

- Background was 118 pmd, so the best signal to noise occurs at 15 min 13.6x backgrou However, by 15 min, incorporation is slowing down as gaps are filled in. At 15 min some turnov is occuring at nicks - not a good model of a PCR reaction * Trade off between good model and s
- Linearity of the time points is good
- A 10% drop in activity would be detected by this assay, using 5 replicates.

POLYMERIZATION: Tfl/VENT



- Incorporation falls off after 5 min, because gaps are filled by high Tfl polymerize activity. After that, turnover occurs at nicks.
- % turnover increases beca DNA synthesis is slowing d while turnover keeps com at the same rate.

Witnessed & Understo d by me,	Date	Inv nt d by	Date
Deborah Bokan	6/26/95	R corded by Pawlyn Combs	6/25/95

ng N ___
units
Turnover
CPM1
= dADP - dATP
= dAMP

	Turnover (pmoles) dAMP $\left(\frac{\text{cpm} - \text{background cpm}}{\text{specific activity}}\right)\left(\frac{20}{10}\right)\left(\frac{100}{2}\right)$	% Turnover (pmoles dAMP / pmoles incorporation + dAMP) x100
1776.00 – 86470.00	ex. $\left(\frac{1776 - 807}{465}\right)\left(\frac{20}{10}\right)\left(\frac{100}{2}\right) = 208$	ex. $\frac{208}{208+936} \times 100 = 18.2$
2994.00 – 86141.00	470	21.2
4209.00 – 91512.00	732	25.1
4983.00 – 85588.00	898	24.7
6822.00 – 94359.00	1290	32.1
7013.00 – 85869.00	1330	33.3
1216.00 – 79679.00	88.0	13.9
2179.00 – 83426.00	295	24.0
2954.00 – 81631.00	462	25.0
3716.00 – 83944.00	623	26.6
4469.00 – 85258.00	788	29.8
5283.00 – 87259.00	963	30.3
1223.00 – 85430.00	89.5	18.7
1807.00 – 90067.00	215	25.2
2316.00 – 88894.00	325	27.5
2953.00 – 84914.00	462	30.6
3572.00 – 90268.00	595	30.2
3815.00 – 92711.00	647	33.0
792.00 – 81173.00		
749.00 – 87079.00		
655.00 – 87371.00		
880.00 – 86383.00		
785.00 – 86929.00		

$\bar{x}$ dAMP = 807 cpm is background = 174 pmoles

specific activity =

$$\frac{929,462 \text{ cpm} \times \frac{100\text{ul}}{10\text{ul}}}{(5000 \text{ pmole})(4)} = 464.7 \frac{\text{cpm}}{\text{pmole at DNA}}$$

CPM1

978.00 – 90674.00

To Page No.____

sed & Understood by m	Date	Invented by	Date
Rebecca Polans	6/19/95		6/19/95
		Recorded by Carolyn Combs	

From Page No. Results: Relative mobility, RM = distance from origen to cente

6/20/95 CSC

	SAM	CPM1	distance (cm) from origen to center of rectangle	↓ RM .45/9 = 0.072
no enzyme, front at 9cm	1	21456.00	0.65	0.17
	2	436.00	~~0.85~~ 1.5	0.21
	3	217.00	~~1.65~~ 1.9	0.26
	4	128.00	2.3	0.30
	5	105.00	2.7	0.41
	6	232.00	3.65	0.54
	7	73.00	4.9	0.66
	8	68.00	5.9	0.77
	9	78.00	6.9	0.91
	10	88.00	8.2	
60° vent, front at 9cm	11	18209.00	same data as above	
	12	572.00		
	13	153.00		
	14	87.00		
	15	175.00		
	16	1759.00		
	17	119.00		
	18	78.00		
	19	151.00		
	20	160.00		
90° vent, front at 9cm	21	17132.00	same data as above	
	22	42.00		
	23	46.00		
	24	99.00		
	25	314.00		
	26	2236.00		
	27	60.00		
	28	72.00		
	29	98.00		
	30	174.00		
no enzyme, front at 13cm	31	21668.00	1	0.077
	32	250.00	2.25	0.17
	33	175.00	2.75	0.21
	34	112.00	3.25	0.25
	35	115.00	3.75	0.29
	36	201.00	5	0.38
	37	63.00	6.5	0.5
	38	71.00	8	0.62
	39	101.00	10	0.77
	40	100.00	12	0.92
60° vent, front at 13cm	41	17710.00	same	
	42	326.00		
	43	133.00		
	44	125.00		
	45	104.00		
	46	1786.00		
	47	59.00		
	48	56.00		
	49	155.00		
	50	121.00		
90° vent, front at 13cm	51	16715.00	same	
	52	400.00		
	53	225.00		
	54	82.00		
	55	91.00		
	56	2316.00		
	57	76.00		
	58	60.00		
	59	100.00		
	60	194.00		
no enzyme, front at 18cm	61	21188.00	1.25	0.
	62	499.00	2.75	0.
	63	195.00	3.25	~~0.~~
	64	86.00	3.75	~~0.~~
	65	83.00	4.25	0.
	66	99.00	4.75	0.
	67	81.00	5.25	0.
	68	75.00	5.75	0.
	69	139.00	7.25	0
	70	73.00	9.25	0.
	71	66.00	11	0.
	72	53.00	13	0.
	73	130.00	~~14~~ 16	
60° vent, front at 18cm	74	19038.00	same	
	75	333.00		
	76	136.00		
	77	79.00		
	78	59.00		
	79	90.00		
	80	59.00		
	81	73.00		
	82	1811.00		
	83	70.00		
	84	50.00		
	85	55.00		
	86	262.00		
90° vent, front at 18cm	87	18132.00	same	
	88	449.00		
	89	249.00		
	90	72.00		
	91	56.00		
	92	76.00		
	93	70.00		
	94	66.00		
	95	2231.00		
	96	99.00		
	97	45.00		
	98	68.00		
	99	234.00		

T Page

Witness d & Underst d by me,	Dat	Inv nted by	Dat
Deeanna Polanco	6/26/95	R c rd d by	6/20/95

Primer degradation by TFl/Vent-Epicenter + Vent alone Pr j ct No._____ B k No._____
Time course + 5 replicates on 12% gel

e N .__

-pose: To measure 3'→5' exonuclease activity of TFl/Vent mix using the primer degradation assay. The data will be part of the stability study on the mix. The following changes to the assay will be made in hopes of obtaining more accurate data on the rate of primer degradation:
i) 12% gel will be used instead of an 8% gel - may give better peak resolution. Last time, peak shoulders and double peaks were a problem during quantitation
2) 44mer Fidel will be used instead of 33mer - correct - the 33mer could form primer dimers &/or a hairpin that may have altered the degradation rate.
3) By doing 5 replicates, we can access the accuracy of this assay as compared to the turnover assay.
Primer degradation by Vent alone will also be measured.

kground: Note - although we tested TFl/Vent mixes on p. 9 NB 11, only the TFl purchased from Epicenter is TFl. LTI's TFl is really Tth. That is why we are not doing any stability tests on LTI's enzyme, until a new TFl alone is obtained.
- the amount of enzyme and time course of this experiment are known to be in linear range of assay from the ealier expt on p. 9 NB 11.

erials: γ^{32}P-end labeled 44-mer Fidel
fresh mix A
12% denaturing sequencing gel
stop soln
TFl (Vent) mix made w TFl purchased from Epicenter 5/3, Vent 10 of #17

or recipe for 12% gel, 100mL: 48g urea
30mL, 40% acrylamide:bis mix
10mL, 10x TBE
dissolve by stirring + low heat
heaped 10% AP (100 mg/mL)
qs to 100mL w/ H_2O - squirt bot.
30µl TEMED
100mL

To Page No.____

I & Understood by me,	Date	Invented by	Date
Clamp	6/26/95	Recorded by Carolyn Combs	6/21/95

Project No.
Book No. TITLE

No.

A: 90ul per rxns, enough for 15/9 rxns

15
66ul x 9 = 594 ul H_2O 990ul
20ul x 9 = 180ul 5x Cheng buffer 300ul
4ul x 9 = 36 ul ^{32}P-44mer Fidel, 5uM stock C.f=200n
90 x 9/15 = 540ul 1350

edure:

label the 44-mer Fidel primer - 70.3ul H_2O
24ul 5X Kinase buffer
6ul 44mer Fidel, 100uM st
13.7ul $^{32}P\gamma$ATP, 10uCi/ul
ref date = 6.
6ul PNK 1u/ul
120ul

37°C, 30min
55°C, 5min
store at -20°C overnight

deliver 5ul Stop soln to 9600 tubes * 1-28 / label rxn tubes 1-
make mix A

rxn #	1	2	3	4	5	6	7	8	9	10	11	12
H_2O	8	8	8	8	8	8	8	8				
mix A	90	90	90	90	90	90	90	90				

preheat to 65°C in 96CC, start by adding e

Ep: 5B * / no enzyme 3ul

2ul TFI / Vent - Epicenter 2ul Vent

At each time pt remove 10ul rxn to 1ul stop soln
- heat 90°C
- spin

no enzyme 0', 10' replicas: 4' 6' at TFI / Vent
time course 2', 4', 6', 8', 10', 20' w/ TFI / Vent

To Page N

& Understood by me,

Dat 6/26/95

Invented by

Record d by Dawlan Pomb

Date 6/21/95

g N. _____

icenter SB for no enz control: final 100mM NaCl, add solid to LTI SB
nd to dilute vent with. final 50mM Tris
now at 20mM

5mL Taq SB = LTI SB ✓
91 ul 1M Tris 7.5 - premade by LTI ✓
62.5 ul 1M Tris-HCl ✓ → 157.649/mole
153.5 ul glycerol ✓
0.031 g NaCl (58.449/m) ✓

$\frac{1\text{ mole}}{L} \times 10\times10^{-3} L = 0.01$ mole

$\frac{g}{157.649/m} = 0.01$ mole

$= 1.5764$ g Tris·HCl^{LTI}
+ 10 ml dH_2O

Vent dilution in Epicenter storage buffer = 22.2x dilution to 0.09u/ul
2 ul Vent stock (2u/ul) w/ p2 pipetman → 1st spin down + vortex Vent stock
+ 42.4 ul Epicenter storage w/ p200 pipetman
44.4 ul vortex to mix

mples were heated to 90°C, 5' in 9600 prior to loading.

sample #'s: 1 = no enz 0 min
2 = no enz 10 min
3 = TFI/Vent 2' min - not preheated = may be off
4 = " 4'
5 = " 6'
6 = " 8'
7 = " 10'
8 = " 20'
9 (1) TFI/Vent 4' → 1
10 " 6'
11 (2) 4' → 2
12 6'
13 4' → 3
14 6'
15 4' → 4
16 6'
17 4' → 5
18 6'
19 Vent 4' → 1
20 6'

21 4' → 2
22 6'
23 4' → 3
24 6'
25 4' → 4
26 6'
27 4' → 5
28 6'

2nd load 1,3-28,12 ~~2nd~~ run longer
2, 3 - 28, 8
1st load 1,2 - 28,2 ~~1st~~
1, 19 - 28, 2 no times course

To Page No. _____

d & Understood by me,	Date	Invented by	Date
Solamp	6/26/95	Recorded by Carolyn Combs	6/21/95

Project No._____

Book No._____ TITLE _____

om Page No.__

sample #

1st loading: 1, 9-28, 2 (no time course) ~3:15 pm, rate 0.7cm / 8 min ≈ 0.1 cm/min

2nd loading 2, 3-28 w/ time course ~5:15 pm, run until expect ch
BØB reaches bottom. At this time ~10 bases will have run
This will be our whole gel loading to see the whole ge
most of the products

Gel was run at 1700V constants for a total of 5.5 hrs.

To Page N

n ssed & Understood by me,	Date	Invented by	Dat
	6/26/95		6/21/95
		Recorded by	

Exhibit 114
Appl. No. 09/558,421

Project

40 Book No. TITLE M13 PCR system: Taq and Tne

From Pag No.

PCR mix:

(40 Rxns)

LTI 10X PCR buffer	400 µl	(1.5
50 mM $MgCl_2$	120 µl	(2.0
4 dNTPs, 10 mM each	80 µl	(1
M13RF 1 pg/µl ▲ see page 42 for dilution	40 µl	
M13-6301 (anchor), 20 µM	80	←
H_2O	3160	(400 primers
	3880 µl	

(18 Rxns)

✳ (in Falcon tube)

1	2
1746 µl	1746 µl
~~H_2O~~	18
rTaq 5 u/µl	20 µl 18 µl
Tne 5 u/µl	
1764 µl	1764

make 2 setups

1-16 gets elongation

1'-16' gets elongation

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

[1] 98 →

[2] 98 →

1 20 µM primer see page 42-43

M13 6671 2
7069
407
806
1491
2506
3972
5464

100 µl

RF

1 pg M13/100 µl
$= 3 \times 10^{-15}$ mole nt
$= 2 \times 10^{-19}$ ~~circles~~ nt

$(2 \times 10^{-19})(6.0 \times 10^{23}$ molecules/

= 125,000 molecules circles

94°C 1 min → 30 cycles: 94°C 15 sec, 55°C 30 sec,

elongation is 6 min for PCR 1-16 and 2 min for 1'-16' → cont. on

T Pag

Witnessed & Understood by m ,	Dat	Inv nt d by	Date
[signature]	6/26/95	R c rd d by	6-23-95

T-RC and Tag with Cheng buffer
in gap DHP

N .

	1	2	3	4
5 x Cheng buffer (no dNTPs) (add P20, 10)	10	10	10	10
10 mM dNTPs	1	1	1	1
Human spleen genomic DNA 20ng/µl	1.25	→	→	→
2112 10µM	1	—	1	—
2113 10µM	1	—	1	—
5 u/µl Taq	0.5	0.5		
			0.5	0.5
Fill 5 u/µl H_2O	35.25	33.25	35.25	33.25
	50µl			

T Page No.

d & Understood by m ,	Dat	Inv nt d by	Dat
B.C.	6/26/15	R cord d by	6-23-15

ng No.__

SigmaPlot regression lines:

1) for Proofreading: Tfl Vent the slope = 28.676 pmole/min, $r^2 = 0.978$

2) for Polymerization: Tfl/Vent the slope = 417 $\frac{pmole}{min}$, $r^2 = 0.873$

Units of Tfl/Vent proofreading: $28.68 \frac{pmole}{min} \times \frac{30 min}{10,000 pmole/2ul} = 0.043 \frac{u}{ul}$

Units of Tfl/Vent polymerization: $417 \frac{pmole}{min} \times \frac{30 min}{10,000/2} = 0.63 u/ul$

expect: 0.045 u/ul

Conclusions

1) The turnover assay can detect a 10% loss of 3' exo activity in Tfl/Vent mixes. By repeating the assay more frequently and/or with more replicates the error may decrease so that a 5% loss of activity could be detected.

2) Early time points, before 10min, reflect turnover during DNA synthesis - the best model of PCR. Later time points reflect turnover during DNA synthesis plus turnover at DNA nicks - not such a good model of PCR. However, the later time points give better data because the signal to noise ratio is higher (3.6x versus ~1.3x early on). Both All 3 time points should probably be done during the stability study.

3) Turnover by Tfl/Vent mix is about 3x higher than by Vent alone. This result was observed in an earlier experiment too (pp40,10). Tfl may create more mismatches for Vent to turnover than when no Tfl is present.

↑ not true: exo in Tfl/vent is only ~ 2x higher than for vent alone (as mistake on p 40,10). In this experiment (pp 7,11) the Vent alone of 2 pl of 0.1 u/pl is the one we should compare to the Tfl/Vent mix

To Page No.

I & Understood by me,	Date	Invented by	Date
[signature]	6/26/95		6/24/95
		Record d by Carolyn Combs	

No.

PLATES

Both the dATP+dADP peak and dAMP peak become more spread out on the PEI plates as the solvent front runs further.
The distance between the two peaks becomes greater as the solvent front runs further: ~0.5cm vs 2cm

Conclusion: For best resolution of dAMP from dATP+dADP on PEI plates, run the LiCl solvent front to the top of the plate, ~16 cm from origen.

To Page No.

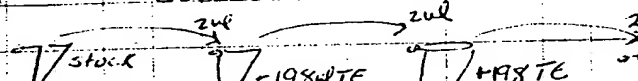
d & Understood by m ,	Date	Inv nted by	Date
[illegible] Polanyi	6/26/95	Recorded by Carolyn Combs	6/24/95

Project No. _____

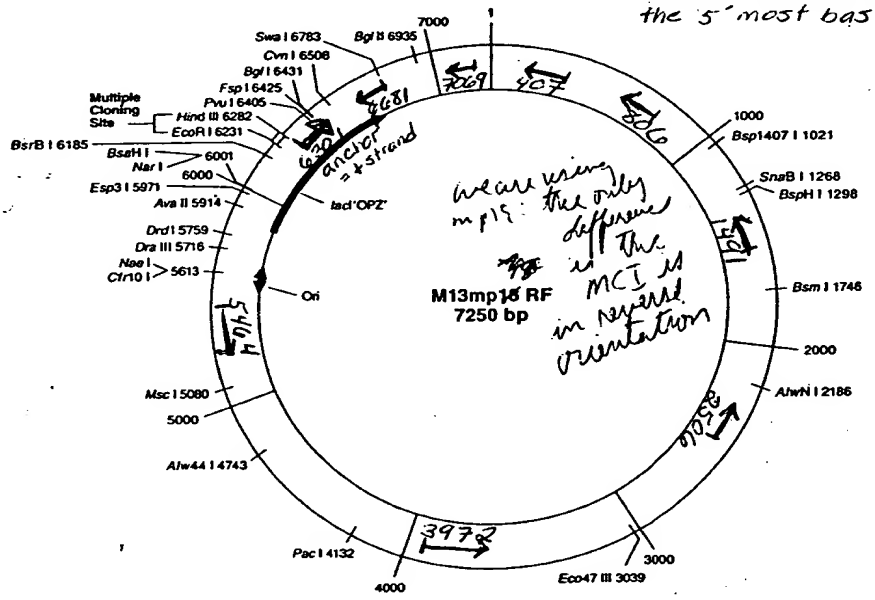
2 Book No. _____ TITLE _____

rom Page No. ___

Dilution of M13 RF to 1 pg/ul in TE: stock lot CN4132, 5ug/18.4ul
do serial dilutions to 1/2.72x10^5



6/20/95



M13mp19 primer

→ 20mers

→ 9 G+C

→ 11 A+T

→ Tm = ~58°C

primer name	PCR product length (bases)	sequence
M13-6301 anchor (=+strand)		5' GTTTTACAAC G TCGTG
M13-6681 (=-strand)	380	5' TTCC TGTAGCCAGC TTTC
M13-7069 "	768	5' ATGCCTGAGTAATGTGTAGG
M13-407 "	1356	5' GAAGCAAAGCGGATTGCA
M13-806 "	1755	5' TTA TACCAG TCAGGACGT
M13-1491 "	2440	5' AGCTTGATACCGATAGTTGC
M13-2506 "	3455	5' CGACAGAATCAAGTTTGCC
M13-3972 "	4921	5' AATCG CAAGACAAAGAACG
M13-5464 "	6413	5' GTATAACGTGCTTTCCTCG

T Pag N

Witnessed & Understood by me, [signature]

Dat 6/26/95

Invented by [signature]

Record d by Pandora Pombo

Date 6/24/95

e No.__

paration of primer stocks and dilutions:
Primers were made by Gibco BRL Custom Primers order# 510790A
Each ~~d~~ primer was ~~dilut~~ resuspended in sterile dH_2O at a CF = 100uM
-spun down, H_2O added, 2min RT, vortex, invert

primer	nmoles/tube	volume of dH_2O added to make CF = 100uM
3-6301 anchor	54.7	547 ul
3-6681	44.09	440.9
3-7069	~~39.14~~ 57.29	~~391.4~~ 572.9
3-407	39.14	391.4
3-806	51.92	519.2
3-1491	69.49	694.9
3-2506	66.34	663.4
3-3972	34.68	346.8
3-5464	42.45	424.5

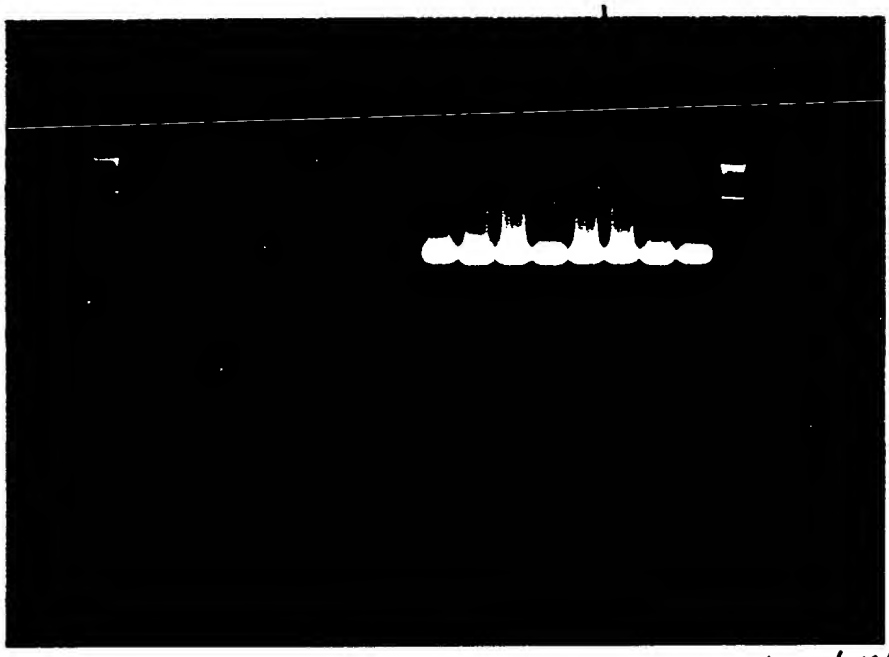
~~Each~~ 20uM aliquots of each primer were made from the 100uM stocks:
⇒ 1:5 dilution, 40ul of 100uM primer stock
+160ul dH_2O-sterile
200ul for each primer except m13-6301 anchor
1:5 dilution of m13-6301 anchor, 200ul of 100uM stock
+800ul dH_2O-sterile
1ml

uation of PCR expt from p 40
2ul of 1Kb ladder and 10-18ul of PCR products were run on a 1% TAE
garose gel at 190V (~180mA, 40W)
o gel recipe: 220ml 1x TAE buffer: 2L 1x TAE
2.2g agarose w/ 70ul EtBr at each end
wt = 435.6g, boil, reweigh & add H_2O back to orig. wt
stir & cool
add 15ul, 10 mg/ml EtBr & pour into rig w/ quarters

To Page No.____

d & Und rst od by m ,	Date	Inv nted by	Dat
olemp	6/26/95	R corded by Carolyn Combs	6/26/95

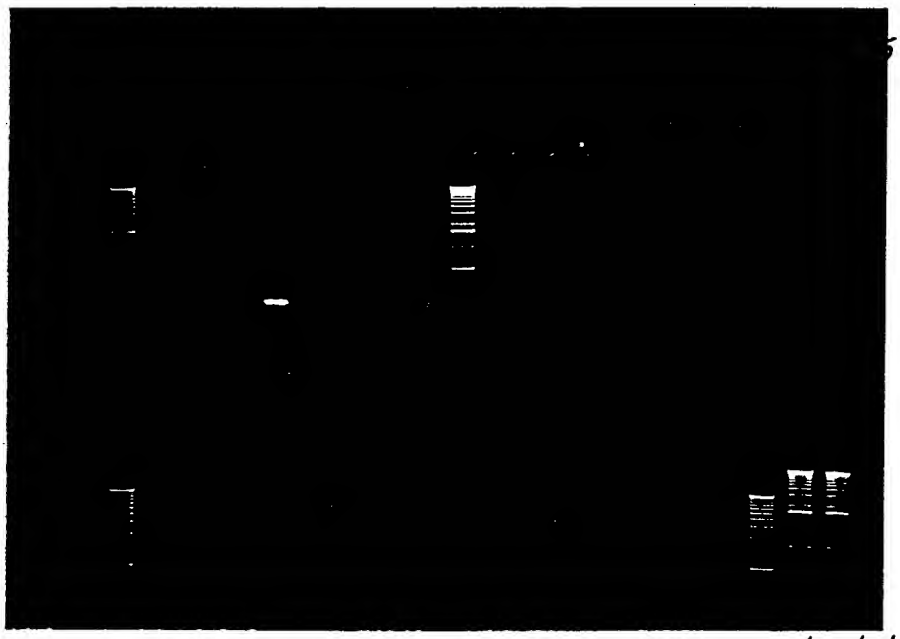
From Page N .__



taped into book 6/26/95 cc

> Since 10ul of the rTa PCR runs contained suc low level of product, tried loading 15ul in or to be able to visualize products better

> No specific products seen in the Tne lath



taped into book 6/26/95 cc

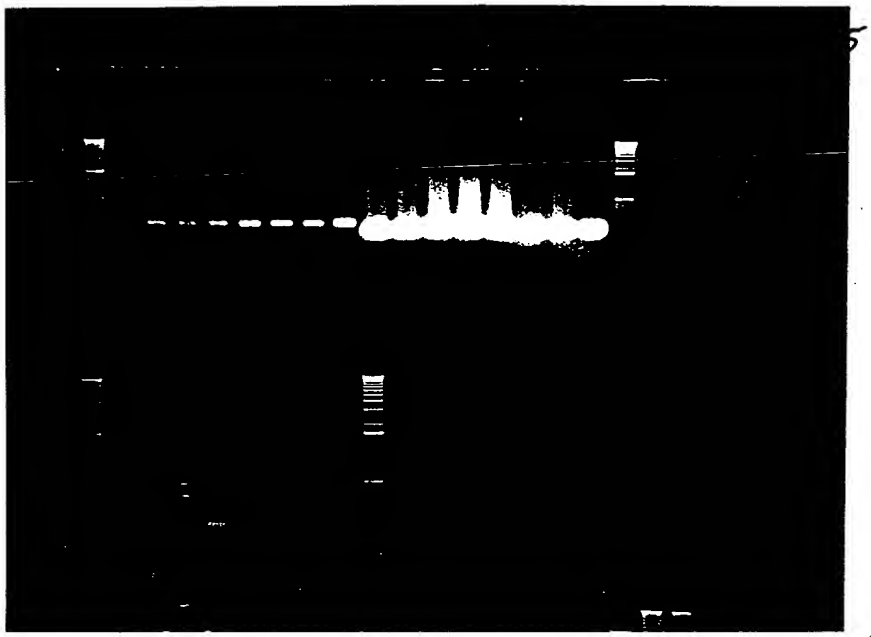
> note false product 1491 primer
- only 1 band of 2. was expected
- instead there are ~0.5-1Kb and ~2-3

> Note false product m13 2506 primer - should be ~3.5Kb
> also a short, false product w/ m13-5464

To Pag

Witn ssed & Und rsto d by me,	Dat	Invent d by	Dat
[signature]	6/30/95		6/26/95
		Rec rd d by [signature]	

g N .



> 6' extention time did not give more product. - worse yield than with 2' extension

cc taped into book 6/26/95

To Page No.

d & Understood by me,	Dat	Inv nt d by	Date
SB Davis	6/30/95		6/26/95
		Recorded by Carolyn Combs	

M13 PCR system: optimizing annealing temperature

g N

	[A]	(12 Rxns)
LTI 10x ~~stn~~ PCR buffer	120 ✓	
50 mM MgCl2	36 ✓	
4 dNTPs 10 mM each	24 ✓	
M13 RF 1 pg/µl (lot# CN4132)	12 ✓	
M13 6301 "anchor" 20 µM	24 ✓	
rTaq 5 u/µl	12 ✓	
H2O	948 ✓	
	1.176 ml	

15 1°

51, 49, 47°C annealing temp w/ 1st 3 oligos program 19, 94°C
18, 94, 15"
- 51, 3"
70, 2"

1 2 3 4 5 6 7 8 9

[A] 97 µl →

	1	2	3	4	5	6	7	8	9
6681	2			2			2			✓
7069		2			2			2	
407			2			2			2

V_f = 100 µl

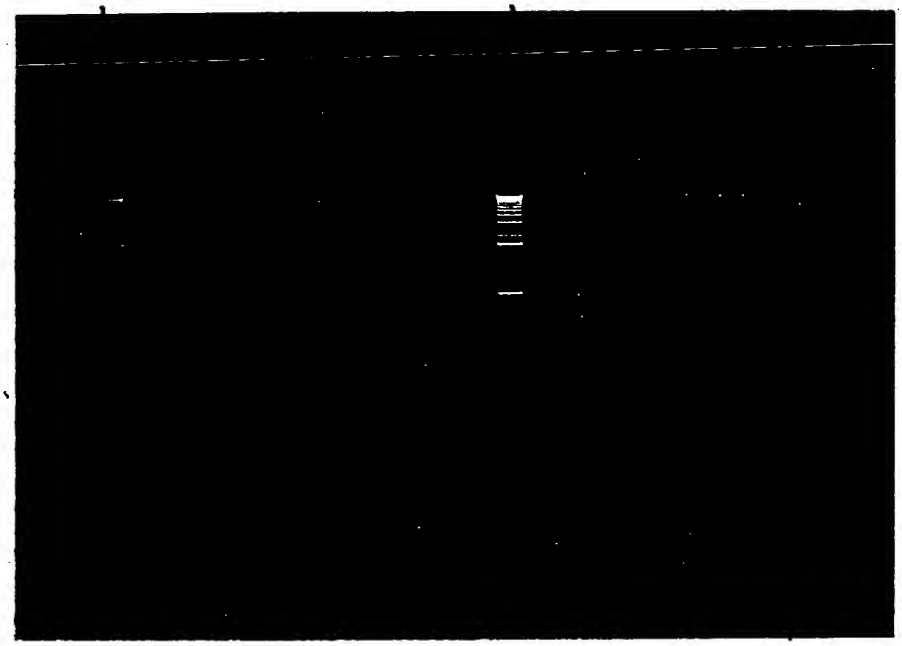
b ~~15~~ 14 9600 51°C annealing temp 1-3
15 49°C 4-6
16 47°C 7-9

% gel: ~~2.64 g agarose + 220 mL~~ ~~220mL~~ 10µl 10Kb ladder, 1 — 9, 10µl 10Kb ladder
3.36 g agarose + 280 mL TAE wt = 480.3 g

To Page No.

...ed & Understood by me,	Date	Invented by	Date
[signature]	6/30/9[illegible]	Recorded by [signature]	6/24/95

Result:



6/20/95 cc

primer combo	expected product length (bp)	observed product length (bp)
anchor + 6681	380	✓ looks like 380 relative
anchor + 7069	768	✓ 768 "
anchor + 8407	1356	✓ 1356

> specific products of the right length were made, but the yield was still v. low. C/c thought lowering the annealing temp might result in a higher yield. It did not. The lanes on the right side of the gel just look a bit darker because the light box is brighter on that side (note how the 10Kb ladder looks more intense on right side even though load was loaded on left & right sides. Also note

> Next will try to increase the yield by using denaturation time (from 15" to 30" - Vern's suggestion), [Taq], & cycle #, [primer] - in case the anchor primer has a hard time annealing due to 2° struc. If it does, than lowering annealing temp would exacerbate the problem.

T Pag N

Witness d & Underst d by m ,	Dat	Inv nt d by	Dat
	6/30/95		6/26/95
		R c rded by	

e N .—

	[A]	10 Rxns
TT 10x PCR buffer	100 ✓	
50 mM $MgCl_2$	30 ✓	
4 dNTPs 10 mM	30 ✓	
413 RT (Lot FAS70) 1 pg/µl opened 6-26-95	10 ✓ →	different lot than on p. 40
H_2O	740 ✓	dilution of stock
	900 µl	stock 370 ug/ml → 2 ul → 198 ul H_2O → 2 ul → 198 ul H_2O → 10 ul → 360 ul H_2O
		1/100, 1/100, 1/37
		= 3.7×10^5 fold dilution

#	1	2	3	4	5	6	7	8
90 µl								
5 µl								
30 Anchor (413-40 anchor)	4	8	4	7	4	7	4	8 ✓
5 q/µl	1	1	2	2	1	1	2	2 ✓

1:1 mix of anchor + 407
30 ul 20 uM stock anchor
30 ul 20 uM 407
60 ul, 10 uM each
(400 or 800 nM oligo each)

94°C, 1' initial denaturation

15" denaturation
94°C
↓
53°C, 30 sec
↓
70°C 2 min
Lab 15, 9600

30 sec denaturation
94°C
"
"
Lab 16, 9600

30 and 40 cycles 3:15 pm
↳ 20 ul rxn 1-8 ↳ left in PCR machine, at 4°C ON
+ 2.3 10x LD
Freeze ON

To Page No.

& Understood by me,	Date	Invented by	Dat
	6/30/95	Recorded by Carolyn Conti	6-26-95

Project No._____ Results P. 49

50 Book No._____ TITLE _____

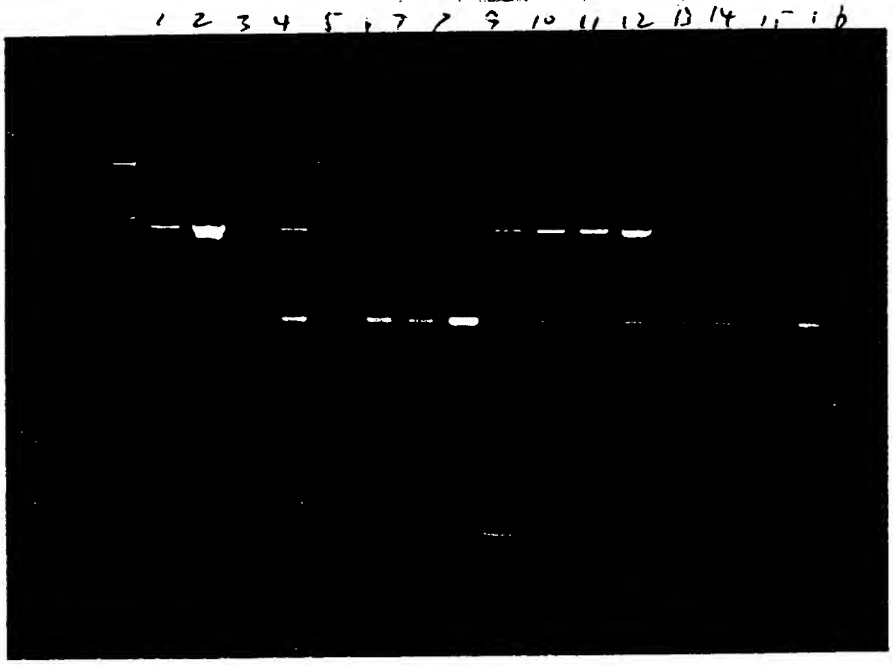
From Pag No.___

Denaturation time: 15 sec | 30 sec

units Taq/100x: 5 | 10 | 5 | 10

primer (nM): 400 | 700 | 400 | 700 | 400 | 700 | 400 | 700

cycles: 30 38 | 30 38 | 30 38 | 30 38 | 30 38 | 30 38 | 30 38 | 30 38



≈ 1356

← primer dimer

0. tube 1 compared to tube 3 on #4 & low # new MJ3RF only gives little or no improvement in yield at 30 cycles
1. lower [primer] (400 nM) is best (tubes 1,2 vs 3,4) (when denaturation is for 30 sec, 400 vs 700 nM primer about equal)
2. Lower Taq is best: only primer dimer made for 10 (#5-8 and 13-16)
3. 38 cycles made more product than 35 cycles (tube 2 vs 1) ag
4. 30 sec denaturation gave less product than 15 sec.

To Pag N

Witn ssed & Und rst od by m ,	Date	Invent d by	Dat
	6/30/95	R corded by Carolyn Combs	6-26-95

g No.

Therefore:

keep 15 sec denaturation

Try even less primer (eg 100 200 300 400 nM)

Try even less Tag (2, 3 4, 5 units)

Try diffent apte number (30 – 40)

Try M13 6301 (another) alone and with other primer with no target to look for primer dimer

Try more M13RF target
eg. 0.1 pmol – 10 n mol

Try R1 (or Bam, HindIII etc) check buffer salt out
PCR with no purification

Test all primers with test conditions

To Pag No.

ed & Understood by me,	Date	Invented by	Date
[illegible]	6/30/09		6-26-09
		Recorded by Carolyn Canby	

TITLE 1.1X PCR stability assays 4°C storage (see P121,9) -20° and -70°C. freeze Thaw

From Pag No.

	Rxn #	assay ul	
# 10 (P121,9): no det	1-3	2	✓
# 11 " 1.1X	4-6	3.64	✓
Tang # 11 : 1.1X	7-9	3.64	✓
Tag 1/125 dil (same as P121,9)	10-14	2	✓
1.1X May 8 1995	15-17	2	✓
1.1X field test	18-20	2	✓
1-27-95 -20°C	21-23	2	✓
-20°C 5/24/95	24-26	2	✓
-70°C 5/24/95	27-29	2	✓
*10 freeze thaw	30 32	2	✓
20 freeze thaw	33 35	2	✓
30 freeze Thaw	36 38	2	✓
For 3-17-95 200 Tne			
P 25, 10			
1/100	39	2	✓
1/800	40	2	✓
1/200	41	2	✓
Tne 5-7-95 (~ 70.7 u/ul on P25)			
1/8000	42	2	✓
	43	2	✓
	44	2	✓

This is 5 month point for 4°C studies (same as P121,9; 154,9; 174,9; 37,10 = 0, 1, 2, 4 m (154,9 is 0 time point, P 38,10 is 1 month)

called "new" on P 34, 10
called "old" P 34

Joe S. took aliquot from samples o on 1-27-95 save 8 freeze thaw stored at -20°C, 0° 5 months at with unknown effect from freeze/thaws

from 1.1X May 8 1995 (compare to Rxn # 15-17 above) 1 month at -20°C with no extra freeze th from 1.1X May 8, 1995 - 1 month at -

* used 1.1X May 8, '95 (above) "new" in drying EtOH → 30°C bath re start with 60 µl and take out at 10, 20, 30 freeze thaw

repeat of P25 for Tne did 5200 fractions look activity at 4°C

47 µl Tag unit assay mix (P120,9) in each
10' 74°C
Kill with 10 µl 0.5 M EDTA, spot 20 µl on G-FC

To Pag

Witnessed & Understood by me, Dat 6/30/95

Inv nt d by Dat 6-28-95

R c rd d by

BEST AVAILABLE COPY

Results P.J 2 — Appl. No. 09/558,421 — Project N ______ Book N ______ 5

No.	Counts		u/l relative to Tag (#10-14)	percent of zero time for P122/9
1	1597.00			
2	1752.00			
3	1760.00			
4	7709.00			
5	6150.00	6760 ave	.030	94%
6	6422.00			
7	4510.00			
8	5085.00		.025	72%
9	5662.00			
10	8620.00			
11	9351.00			
12	8531.00	8931 CPM ave	.04 (by definition)	
13	8321.00			
14	9832.00			
15	5618.00			
16	5895.00	5632 ave $\frac{5632}{8931}$ .04	.025 $\frac{.025}{.023}$	109%
17	5384.00			
18	5128.00			
19	5036.00	5215 ave $\frac{5215}{8931}$ (.04) =	.023 u/l $\frac{.023}{.023}$ ≠ 100%	
20	5481.00		↑ P34.95 for start	
21	3989.00	its been at -20°C for ~5 months + 4 Freeze thaws		54% recovery from .05 u/ul measured above (#4-6)
22	4058.00	3673 ave it came from tube #11		
23	2971.00	above (Ques #4-6)		
24	5931.00	conclude -20°C loses activity		
25	5591.00	5921 → 1 month -20°C from 5-8-95 1.1X (#15-17 above)		
26	6242.00			
27	5891.00	sample	no activity lost	
28	5381.00	5712 from 1.1X of 5-8-95	for 1 month at -70°C	101%
29	5865.00	(see tubes 15-17 above) ⇒		
30	5644.00			
31	5407.00	5440 started with 1.1X		97%
32	5271.00	stored only at 4°C)	conclude no	
33	5362.00	5405 (stored 5-8-95 (see	loss of units	96%
34	5494.00	#15-17 above)	for even	
35	5361.00		30 Freeze Thaws	
36	5556.00	5138		100%
37	6159.00			
38	5200.00			
39	138.00	The died at 4°C of 5200 col of 5-18-95		
40	287.00	got 7.7 u/l on P.25		
41	137.00			
42	1014.00	ave - BKGD		
43	960.00	= 627cpm = 27.3pmol 32.8 u/ul agrees with 36 u/l		
44	1092.00	Lig Flynn got and I got P.13, My 70.7 u/l		
45	395.00	BKGD of P25 may be wrong, will conclude Lig's		
46	110131.00	2λ mix — The is stable until more data is available		
47	110429.00	2λ mix 68.9 CPM/pmol		

To Page No. ____

Read & Understood by me, [signature] Date 6/30/95

Invented by [signature] Date 6-30-95

Recorded by — 6-28-95

Project No.

May 8, 95

(1) 11 sample original bff. formulation

(2) Date field test

(3) (="new" on P34,10) 500ml batch lot

3 batch's

created → 4°C, RT, -20°C 27/1 (8 + thaw)

created → RT, (4°C)

created → 4°C (24/5/95 aliquoted 1-2 ml) → -20°C, -70°C, RT, 4°C

RT & NaN_3 + 1.1 + [illegible]

4 thaws: make aliquots 20 µl each at -20°C (only 1 or 2 freeze thaw)

The unit assay

notes from Joe Solus

6-27-95

Turnover by TfI/Vent - 7 replicates of Epicenter's TfI/Vent and LTI's TfI/Vent in Epicenters SB - zero time at for stability

g N.—

pose: To establish the 3'exo activity of TfI/Vent by Turnover on gapped DNA at time zero of stability study. 7 replicates to reduce error

kground: The zero time point turnover assay has already been for Vent alone and LTI's TfI/Vent in LTI SB with 5 replicates NB 11 page 17 and 21

pio to deliver 10ul stop & 10ul run-wipe tips

ials: mixA, enough for 40 rxns, each using 98ul of mix A for 23 = 1467ul H_2O ✓

40 x 63.786	= 2551.44ul	H_2O	460 5x Cheng ✓
40 x 20	= 800ul	5x Cheng	310ul act DNA
40 x 13.5	= 540ul	activated DNA	11.5 dATGC-TP
40 x 0.5	= 20ul	dATGC-TP, 10mM each	4.92ul α^{32}P dATP
40 x 0.214	= 8.56ul	α^{32}P dATP	2253.422 6/30 AMC
	3920ul		

s	~~1-7~~	8-14	15-21	~~22-28~~	29-35
ube	~~1-21~~	~~22-42~~	43-63	~~64-84~~	85-91
		1-7	8-14		15-21
		1-21	22-42		43-49
A	98ul	prewarm	to 68°C		

Vent (LTI SB) ~~2ul~~
Vent (epi SB) 2ul
/Vent (epicenter TfI) 2ul
~~t diluted~~ (5-14-95) ~~2ul~~
enz 2ul LTI SB = Taq SB

To Page No.____

ed & Understood by m ,	Date	Invented by	Dat
	6/30/95	R corded by Carolyn Coats	6-29-95

From Pag No.

At 5, 10, 15 min remove 10/20ul rxn to 10/20ul stop soln (p.14)
Spot 2ul on PEI plates
Spot 10ul on GFC filters (2 per rxn) + 2ul mix A 3x

tube #5

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18
19	20	21

} epicenter's Tfl enzyme + Vent in ~~LTI~~ epi SB (=Tth?)

22	23	24
25	26	27
28	29	30
31	32	33
34	35	36
37	38	39
40	41	42
5	10	15
~~43~~	~~49~~	

} LTI's Tfl enz in ~~SB~~ epicenters SB + Vent = Tth

43-49 = no enzyme

GFC's
1-6 = 2 replicates of epicenter enz + Vent
7-12 = 2 replicates of LTI's enz in epicenter SB
13 = no enz
16-18 = 2ul mix A

T Pag

Witness d & Understo d by m , Date 6/30/95 Invent d by Date 6-29-95
R c rded by

From Pag No. see p. 51

purpose: The PCR worked well (gave a large amount of produc
when 38 cycles were done using 400nM primer
5 u/100ul Taq
15 sec denaturat

Now we'll try to get the same good plateau yield by optimizing [primer], [enzyme], checking for anchor probe [target] & linearizing target - using just 30 cycles

program 74 Jul X

expt 1. Δ [primer] from 100, 200, 300, 400 nM w/ 5u Taq, 15" denat 30 cycles

make A: 120ul 10X LTI PCR buffer
36 ul 50mM $MgCl_2$
24 ul 10mM 4 dNTP
12ul 1 pg/ul M13RF → dilute w/ TE
888ul H_2O
1080ul

rxn	1	2	3	4
A	90	90	90	90
H_2O	9	8	7	6
primer mix				

A: 50ul 10x PCR buffer ✓
15 ul 50mM $MgCl_2$ ✓
10ul 10mM 4 dNTPs ✓
5 ul 1 pg/ul M13RF ✓
395ul H_2O ✓
5 ul rTaq, 5u/ul
480ul

2ul of 370 ng/ml stock + 198ul TE → mix
2ul + 198ul TE → mix
10ul + 360ul TE ⇒ 1 pg/ul ✓

make primer mix: 6 ul 20uM anchor primer ✓
6 ul 20uM 407 primer
12ul 10uM each

To Pag

Witnessed & Understood by m,	Date 6/30/95	Invented by	Date 1-25-95
		Recorded by	

g No.__ Tube	1	2	3	4
O	0	1	2	3 ✓
mer mix	4	3	2	1 ✓
]	96	96	96	96 ✓

aq] from 1 to 7 units / 100ul rxn → 400nM primers

: 80ul 10x PCR buffer ✓ dilute rTaq 5x
24ul 50mM $MgCl_2$ ✓ 6ul rTaq 5 u/ul ✓
16ul 10mM 4 dNTPs ✓ +24ul Taq dilution buffer ✓
8ul 1 pg/ul M13RF ✓ 30ul
16ul 20uM anchor primer ✓
16ul 20uM 407 primer ✓
584ul H_2O ✓
744ul

2x ✗	5	6	7	8	9	10	11
3]	93 →						✓
H_2O	6	5	4	3	2	1	0 ✓
rTaq 1u/ul	1	2	3	4	5	6	7 ✓
	100ul						

target DNA, 800nM primer, 10u Taq:

2] : 35ul 10x PCR buffer ✓
10.5ul 50mM $MgCl_2$ ✓
7ul 10mM 4 dNTPs ✓
262.5ul H_2O ✓
7ul rTaq (5u/ul) ✓

✗	12	13	14
er	4ul anchor	4ul 407	4ul anchor ✓
)	94	4	0 ✓
	92	92	92 ✓

primers are 20uM

To Pag No.____

d & Understood by m ,	Date	Invented by	Dat
Roland	6/30/95		6-22-95
		Recorded by Carolyn Coles	

From Page No.

Δ [Target], use 400nM primers and 5u Taq / 100ul

for 7 rxns

[D]: 70ul 10x PCR buffer ✓
21ul 50mM $MgCl_2$
1.4ul 10mM 4 dNTPs
1.4ul 20uM anchor
1.4ul 20uM 407
462ul H_2O
7ul +Taq 5u/ul

* dilute m13 RF to 0.5 pg/ul

20ul 1 pg/ul m13 RF
20ul TC

Tube #	15	16	17	18	19	20
m13RF (0.5 pg/ul)	1	1.5	2	6	10	14
H_2O	13	12.5	12	8	4	0
[D]	86 →					

RI digestion of template:

3ul 1 pg/ul m13 RF
5ul 10x PCR buffer
3ul 50mM $MgCl_2$
13ul H_2O
1ul EcoRI 10u/ul
25ul

37°C, 30'

8.3ul 8.3ul 8.3ul
91.7ul E →

3ul
5ul
3ul
13
1ul H_2O

37°C 30' 1 pm – 1.50 pm

same

21 22 23 24 25 26

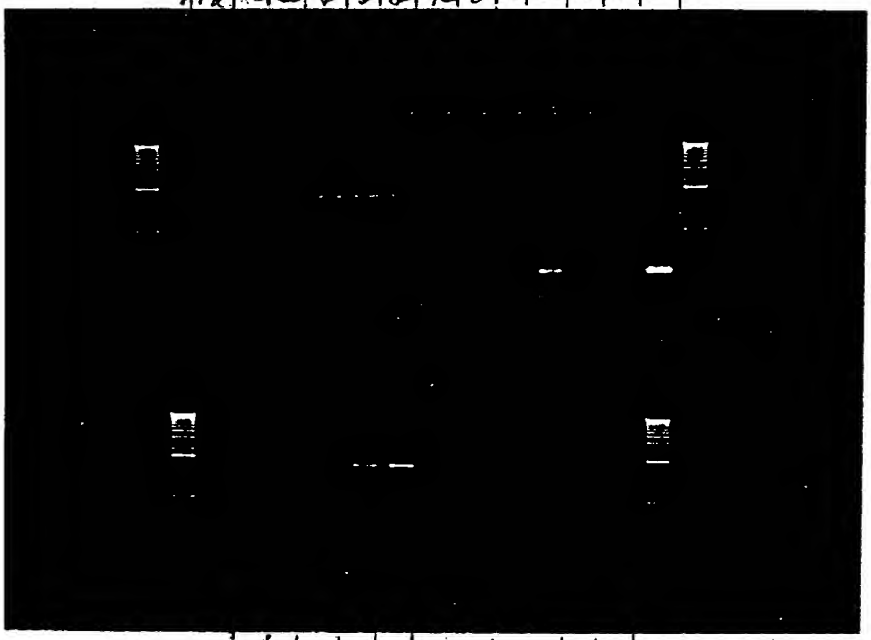
To Pag

Witn ss d & Understo d by me,	Dat 6/30/95	Invent d by	Dat 6-29-95
		R cord d by	

e No.___

4.6 ul	10x PCR buffer	x 7	=	32.2 ✓
2 ul	10mM 4dNTPs		=	14 ul ✓
1 ul	M13RF 1pg/ul		=	7 ✓
83.1 ul	H_2O		=	581.7 ✓
1 ul	Taq 5u/ul			7 ✓
91.7 ul				641.9

anchor + 407 are primers



To Page No.____

I & Understood by me,	Date	Invented by	Date
Olaynes	6/30/95	Recorded by Carolyn Lones	6-25-15

Proj ct No._____

Book No._____ TITLE _____

From Page No.___

Results -

1. Taq is inhibitory. Best results at 1 and 2 units per 100 µl PCR.

2. Yields improve with increasing target

3. Both primers ~~must be~~ are required to make primer dimers

2° ~~It's not~~ hairpin substrate or one primer ~~self anneals~~ anneals to another copy of the same primer.

conclude the anchor primer should be OK for most PCR's

T Pag N

Witn ssed & Underst d by m ,	Dat 6/30/95	Inv nt d by	Dat 6-2595
		R c rded by	

23mer · mp19 ssDNA

e N. _ (see P17, 9)

43 mp19 0.26 μg/μl — 200 μl — Exp. (158 n mol nt total)
79 n mol nt/μl
109 p mol circle/μl — 21.7 p mol circle total
0.594 n mol nt/μl
43.5 p mol primer total

23 mer 5 ng/μl — 66.1 μl
.66 p mol primer/μl)

266 μl

23 mer / mp19 = 2 circles

70° C, 5 min

cool at room temp 40 min

(for 60λ 23·mp19)
conc in 100 μl Rxn

mp19 is 10 mM Tris pH 7.4
5 mM NaCl
0.1 mM EDTA

6 mM Tris pH 7.4
3 mM NaCl
.06 mM EDTA

with Chem 1X (P20, 10) =

20 mM Tricine pH 9
5 mM KOAc
2% DMSO
1.5 mM MgOAc
5% glycerol

∴ buffer in 23·mp19 will alter the reaction conditions a little since 60 μl 23·mp19 is needed per 100 μl Rxn

in future need more concentrated DNA

To Page No. _____

& Understood by me,	Date	Invented by	Date
Bolano	6/30/94	R corded by Carlson Cmt	6-29-95

Exhibit 120
Appl. No. 09/558,421

Project N .____
Book N .____

5:

g N .__

	(a)	u/l relative to Tag (#10-14)	Percent of Zero time pt on P122/9
1	1597.00		
2	1752.00		
3	1760.00		
4	7709.00		
5	6150.00	.030	94%
6	6422.00		
7	4510.00		
8	5085.00	.025	72%
9	5662.00		
10	8620.00		
11	9351.00		
12	8531.00	.04 (by dilution)	
13	8321.00		
14	9832.00		
15	5618.00		
16	5895.00	.025	.025/.023 109%
17	5384.00		.023/.025 ≠ 100%
18	5128.00		↑ P54 J for intent
19	5036.00	.023 u/l	
20	5481.00		
21	3989.00		
22	4058.00		
23	2971.00		
24	5931.00		
25	5591.00		
26	6242.00		
27	5891.00		
28	5381.00		
29	5865.00		
30	5644.00		
31	5407.00		
32	5271.00		
33	5362.00		
34	5494.00		
35	5361.00		
36	5556.00		
37	6159.00		
38	5200.00		
39	138.00		
40	287.00		
41	137.00		
42	1014.00		
43	960.00		
44	1092.00		
45	395.00		
46	110131.00		
47	110429.00		

6760 ave

7931 CPM ave

5632 ave (5632/7931 = .04)

5215 ave (5215/7931 (.04) = .023 u/l)

3673 ave — it's been at -20°C for ~5 months + 6 Freeze Thaws it came from tube #11 above (lines 4-6)

5921 → concluded -20°C loss of activity 1 month -20°C from 5-8-95

5712 — sample from 1:1X of 5-8-95 (same tube as 15-17 above) ⇒ no activity lost for 1 month at -70°C

5440 — started with 1:1X (stored at 4°C) (stored only at -70°C) (see #15-17 above)

5405

5158

concludes no loss of units even for 50 Freeze Thaws

97%
96%
100%

TNC died at 4°C w/ 500 col of 5-18-95 got 7.7 u/l on P.25

ave-BKGD = 62 CPM = 27.3 pmol

32.8 u/ml

agrees with 36 u/l

Eg Flynn got 11 and I got P.13. My 70.7 u/l of #25 may be wrong

BKGD 2 x min

2 x min

TNC is stable

68.9 CPM/pmol

will conclude Eg's until more data is available

54% recovery from .054 u/ml measured above (#4-6)

101%

To Page No.____

ed & Understood by me,	Date	Invent d by	Date-30-95
	6/30/95	R corded by	6-29-95

Project No.

May 8, 95

3 batch:

① 15 sample — orig. buff. formulation
created → 4°C / RT / −20°C (Date 27/1)
4°C → * RT + NaN3 ; * → (1.1x + [illegible] det.) *
−20°C → 9x thawed *

② Date — Field test
created → RT / (4°C) *

③ (= "new" on P34, 10) 500ml batch lot
created → 4°C (24/5/95) aliquoted 1-2ml
→ −20°C * / −70°C * / RT / 4°C *

thaw → make aliquots 20 µl each at −20°C (only 1 or 2 freeze thaw)

The unit [illegible] *

notes from Joel Jolas

R [signature]
6-27-95

ag N .— specific activity = (109,973 cpm) x 100ul rxn / 2ul spot = 275 cpm / pmol (nt)

background = 188 cpm (5000 pmol) 4

Turnover (pmol) = ((cpm − background cpm) / specific activity) (100/2)(20/10)

	CPM1	
5'	774.00	774−188/237 275 x 100 = 228 213
10'	1379.00	433
15'	2170.00	721
5'	1031.00	307
10'	1588.00	509
15'	2241.00	747
5'	893.00	256
10'	1731.00	561
15'	1890.00	619
5'	788.00	218
10'	1365.00	428
15'	1836.00	599
5'	752.00	205
10'	1055.00	315
15'	1732.00	561
5'	636.00	163
10'	1140.00	346
15'	1448.00	458
5'	854.00	242
10'	1458.00	462
15	2083.00	689
	829.00	233
	1512.00	481
	2124.00	704
	980.00	288
	1612.00	518
	2249.00	749
	1182.00	361
	2028.00	669
	2271.00	757
	1040.00	310
	1816.00	592
	2521.00	848
	944.00	274
	1729.00	560
	2032.00	671
	1087.00	327
	1641.00	528
	2701.00	914
	917.00	265
	2146.00	712
	2530.00	851

$\bar{X}$ 5' = 229 ± 45 (20%) → Large error, next time cut out dATP+dADP spot in order to correct for spotting error

$\bar{X}$ 5' = 293 ± 45 (15%)
10' = 580 ± 84 (15%)
15' = 785 ± 88 (11%)

195.00		
162.00	47	186.00
169.00	48	189.00
194.00	49	223.00

no signal

background for turnover
n = 7 $\bar{X}$ = 188.3 ± 19.8 (~10% error)
= 0.8 pmoles

To Pag No.____

sed & Und rstood by m ,	Date	Invented by	Date
Dolan	6/30/95	Recorded by Carolyn Combs	4/30/95

[redacted]

Exhibit **122**
Appl. No. 09/558,421

Project ______ Book No. ______ TITLE Turnover of Tfl/Vent on 23. mp

From Page No. ___ adjust Vf back to 266 λ

	mix A		(5.4 Rxns)
23. mp19 0.~~164~~ 195 μg/μl 0.594 nmol nt/μl P. 63	~~264 μl~~	264 ✓	23.7 ~~29~~ nmol nt * ~~20.2~~ μg/ml 65.6 * see P65 ... change ... of DNA ...
5x Cheng (P21, 10)	~~88 μl~~	108 ✓	
4 dNTPs 10 mM each	~~2.2 μl~~	2.7 ✓	(50 μM each)
α ^{32}P dATP 10 mCi/ml (Amersham)	~~1 μl~~	1.23	
H2O	~~78 μl~~	153.3 ✓	
	~~431.2~~	529.2	(use 98 μl / 100 μl)

see p. 65

Reactions

stop tube *	① 1-8	② 9-16	③ 17-24	④ 25-32	⑤ 33-40 (no enzyme)
mix A	98 μl →				
Tfl/Vent = 0.18 units (Epicentre Tfl 5-16-95)	2				2 μl Epicentre storage buffer
Vent lot #17 = (opened 2-24-95)					
0.09 u/μl = 0.18 u		2			
0.5 = 1 u			2		
2 (no dilution) = 4 u				2	

Vf = 100 μl

note: ... to Vent ... with gap... on P 28

} dilute ... Epicentre Tfl storage buffer

68°C in 9600, remove 8 μl to 8 μl "stop" Killing solution P. ... spot 2 μl on PET and 10 μl on GFC, 13

* note ~~20~~ ~20 μg/ml 23.mp19 is ~1/6 as much total DNA as gapped DNA in Taq unit assay (500 μg/ml) however M13 is almost all ssDNA substrate while gapped DNA may have ~20 ssDNA gaps.

at 5 10 20 40, 60, 80, 100, 120 min

* spot DuPont on PEI

To Page

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	6/30/95	[signature] Recorded by [signature]	6-30-95

Exhibit **123**
Appl. No. 09/558,421

Project No. Results of P.64

Book No.________ **TITLE** turnover on M13SS DNA

From Page No.___

	min		Turnovers	pmol (−2840 nm / 8 kd)	percent turnover	percent incorporation (100)(nmol incorporation / nmol input DNA)
center Tfl / vent	5	1	445.00	83	1.4	24
	10	2	1588.00	672	7.8	33
	20	3	3904.00	1866	13.6	50
	40	4	6787.00	3350	22.4	49
	60	5	6818.00	3368	21.3	52
	80	6	7009.00	3466	21.4	54
	100	7	7002.00	3462	22.4	50
	120	8	7164.00	3546	21.4	55
18 u vent	5	9	333.00	25	—	0
	10	10	350.00	34	—	0
	20	11	381.00	50		0
	40	12	521.00	122	37	0.9
	60	13	832.00	282	43	1.6
	80	14	1097.00	412	45	2.2
	100	15	1474.00	615	49	2.7
	120	16	1928.00	847	53	3.2
1 unit vent	5	17	296.00	6	2	1.0
	10	18	508.00	115	16	2.6
	20	1	1322.00	535	25	6.8
	40	2	3242.00	1524	32	13.8
	60	3	6116.00	3006	42	17.6
	80	4	8505.00	4238	45	22.1
	100	5	11510.00	5787	52	22.9
	120	6	13872.00	7004	52	27.3
4 units vent	5	7	750.00			
	10	8	1728.00			
	20	9	4617.00			
	40	10	9106.00			
	60	11	11531.00			
	80	12	12228.00			
	100	13	12432.00			
	120	14	11890.00			
no enzyme	5	15	269.00	274 ave dAMP background		
	10	16	278.00			
	20	17	240.00			
	40	18	276.00			
	60	19	293.00			
	80	20	274.00			
	100	21	307.00			
	120	22	331.00			

note: it app[ears] there is no T/O at c[...] since T/O stops [when] incorporation stops ~ 20 min

note turnover % low and unlike [...] for Tfl/vent. conclude most are not full [...] since low per[cent] incorp.

← about 25 max incor[p] 13.8%/5[...] (55% seen [...] at 120 min) so still mus[t] are not full

more conclusions

1. T/O is occurring at an e[...] high rate (~ 20-22%) for Tfl/[vent] considering ~ 30:1 Tfl/vent (in units)

2. it appears that substrates a[...] 3' exo accumulate during time [...] probably at hairpins (eg M13 o[...]) rather than at full length [...] (since T/O stops shortly after incorporation stops even for [...])

3. Incorp plateau for Vent alone (~27% incorp) is lower than for Tfl/Vent (~55%) [...] vent gets stuck at hairpins?

T[o] Pag[e] N[o.]

Witness[e]d & Understo[o]d by m[e], | Date 7/7/95 | Inv[e]nt[e]d by [signature] | Dat[e] 7-1-95

R[e]c[o]rded by [signature]

[signature] Polaya

Results of P64

Project No. (23.7 n mol nt input ss M13 DNA)

Book No. Percent substrate copied

67

No.	Incorporation	p mol
23	632.00	
24	37364.00	
25	37879.00	
26	106114.00	
27	104061.00	
28	34.00	
29	57217.00	5898
30	76607.00	7898
31	114434.00	11797
32	112213.00	11567
33	120505.00	12423
34	123172.00	12698
35	115839.00	11942
36	125999.00	12990
37	1441.00	96
38	600.00	(90)
39	1390.00	90
40	2498.00	255
41	4176.00	378
42	5526.00	513
43	6777.00	643
44	7820.00	755
45	2449.00	252
46	6061.00	624
47	15599.00	1608
48	31777.00	3276
49	40517.00	4177
50	50876.00	5245
51	52552.00	5417
52	62653.00	6459
53	36563.00	
54 #38	~~260.00~~	
55	83243.00	8582
56	58704.00	6053
57	82715.00	8527
58	73558.00	7585
λ	69056.00	7119
2	65008.00	6701
60	502.00	
61	591.00	
62	450.00	
63	960.00	
64	307.00	
65	563.00	
66	463.00	
67	80862.00	
68	75044.00	
69	81620.00	

260

-512 BKGD

ave BKGD = 512

ave 7917 ⇒ 194 CPM/p mol nt

To Pag No.

ed & Understood by me,	Date	Invented by	Date
Olaup	7/7/95	R corded by Carolyn Combs	7-7-95

M13 PCR system: more optimization of [primer 407], annealing temp, and [target]

g N ____

A, enough for 28 rxns: 280 ul 10x PCR buffer ✓
84 ul 50mM $MgCl_2$
56 ul 10mM dNTPs ✓
2094.4 ul H_2O ✓
5.6 ul [Taq] 5u/ul ✓
2520 ul

template dilution:
to 50 pg/ul = 1 ul stock M13 RF 370 ug/mL
+ 99 ul TC
mix ✓ ✓
→ 1 ul + 73 ul TC ✓
→ 74 ul of 5 pg/ul
→ to 10 pg/ul →
8 ul + 32 TC ✓
40 ul 10 pg

15 53° 10:50 am
16 55° 11:02 - 1:15
SGI 57°

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

1 - 3 4 - 6 7 - 9

3 RF: 3 ul of 10 pg/ul | 9 ul 10 pg/ul | 6 ul of 50 pg/ul ×

mer mix: 12 ul 18 24 | 12, 18 ul 24 | 12, 18, 24 ul ✓
um anchor
um 407
anchor 407
0: 15 ul 9 ul 3 ul | 9, 3 ul 0 | 12 ul, 6 ul, 0 ul ✓

A: 270 ul → | 270 ul → | 270 ul →
300 ul →

divide into 3 tubes, 1 for each annealing temp. a, b, c

= 53° 10 pg M13RF 4 a 53° 30 pg M13 7 a 53° 100 pg M13
55° w/ 400nM primers b 55° 400nM primers b 55° 400nM p
57° c 57° c 57°

= 53° 10 pg M13RF 5 a 53° 30 pg M13 8 a 53° 100 pg M13
55° w/ 600nM primer b 55° 600nM p b 55° 600nM p
57° c 57° c 57°

53° 10 pg M13RF 6 a 53° 30 pg M13 9 a 53° 100 pg M13
55° w/ 800nM primers b 55° 800nM p b 55° 800nM primers
57° c 57° c 57°

T Pag No. ____

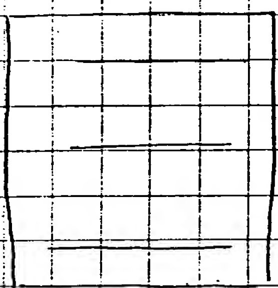
d & Und rstood by me, Polymp
Date 7/7/95
Inv nted by
Date 7/5/95
R corded by Carolyn Combs

Project No._______
Book No._______ TITLE __________

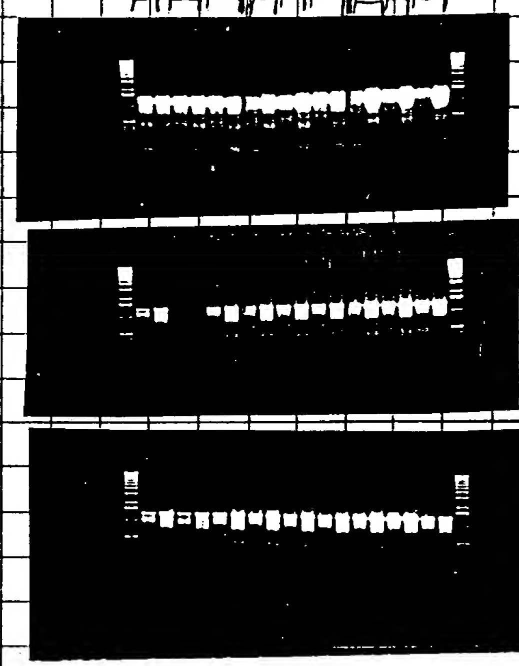
From Page No.__

1% agarose gels: 6 g agarose
600 mL 1x TAE
~~30~~ 4 ul 10 mg/ml EtBr

M13RF: 10 pg | 30 pg | 100 pg
primer (nm) 400 600 800
cycles 30 35



top
57°C C 1-9
55°C B 1-9
53°C A 1-9
bottom



Conclusions

1. no advantage to >400 nM primer
2. more target improves yield and specificity
3. 57°C is most specific

Carolyn Combs 7/6/95

To Page

Witnessed & Understood by me, D Polaro | Date 7/7/95 | Invented by [signature] | Date 7/5/95
Recorded by Carolyn Combs

age N ___

g target

primer (n M)

cycle #

10 30 100

400 600 800 400 600 800 400 600 800

30 35 30 35 30 35 30 35 30 35 30 35 30 35 30 35 30 35

annealing temp

[redacted]

57 °C

55 °C

55 °C

To Page No.___

ed & Understo d by me,

Date 7/7/95

Invented by

R corded by

Date 7-6-95

title: m13 PCR system: 4 annealing temperatures and 4 primer sets
(53°C, 55°C, 57°C, 59°C) (anchor + 6681, 7069, 407, 806)

purpose: We have established optimal [template] & [primers] for anchor + 407. Now we'll optimize annealing temp and cycle # for the other primers 6681, 7069, 806. ~~+ redo 4~~ assuming that these other primers will work well with the [template] & [primer] that worked well w/ 407. Later, we'll titrate [Taq] & [Tne] for these primers.

background:
- 59°C has not been tried before w/ any primers
- in an earlier expt 57°C worked best for 407
- [template] = 100 pg / 100ul rxn - found to be best for 40
- [primer] = 400 nM - found to be best for 407
- [Taq] = 1u/100ul rxn, more was inhibitory for 407
  2u/100ul rxn will be tried for 806 which me
  the longest product
- expected product sizes:

anchor + 6681 → 380 bp
+ 7069 → 768 bp
+ ~~356~~ 407 → 1356 bp
+ 806 → 1755 bp

Carolyn 7/6
Comfs

materials: m13RF 50 pg/ul, diluted on: ~~7/6~~ 7/6/95 NB11 p.

To Pag

Witness d & Understo d by m, Dat 7/7/95
Invented by Date 7/95
R c rded by 7/6/95

ocedure:

ake a master mix for 21, 100ul rxns - containing everything but the primers:

(A)
- 210ul 10x PCR buffer
- 1654.8ul H_2O
- 63ul 50mM $MgCl_2$ C_f = 1.5mM
- 42ul 10mM dNTP's C_f = 200uM
- 42ul m13mp19RF 50pg/ul stock } C_f = 100pg/100ul rxn
- 42ul anchor primer, 20uM stock C_f = 400nM
- 4.2ul Taq 5u/ul C_f = 1u/100ul rxn

2058ul

remove 441ul and add 0.9ul Taq (5u/ul) - for 806 primer w/ 2u Taq/100ul rxn

s (1.5mL)	1	2	3	4	5
ner, 20uM stock	8ul 6681	8ul 7069	8ul 407	8ul 806	8ul 806
	392ul				(B) →
	400ul				

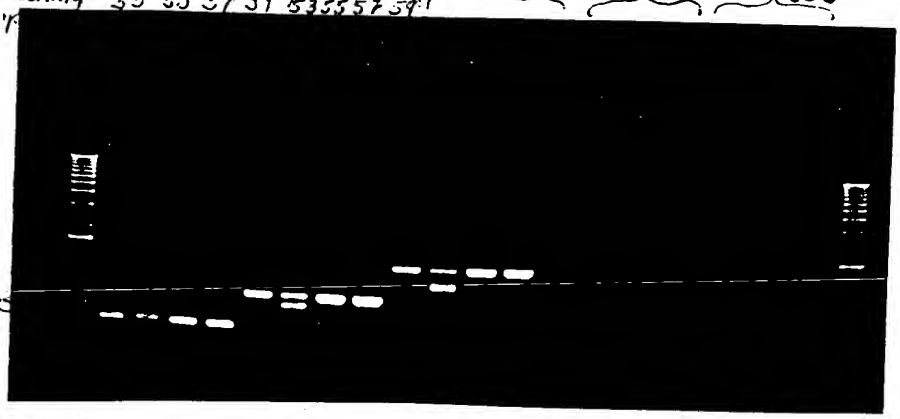
ivide into 4, 100ul aliquots in 9600 PCR tubes nd put each tube in different 9600's set to different nnealing temperatures
- 53° - Lab 15
- 55°C - Lab 16
- 57°C - SGT
- 59°C - Lab 14 * note that 30 cycle aliquots where taken during ramp to 94°C

Carolyn Combs 7/6/95

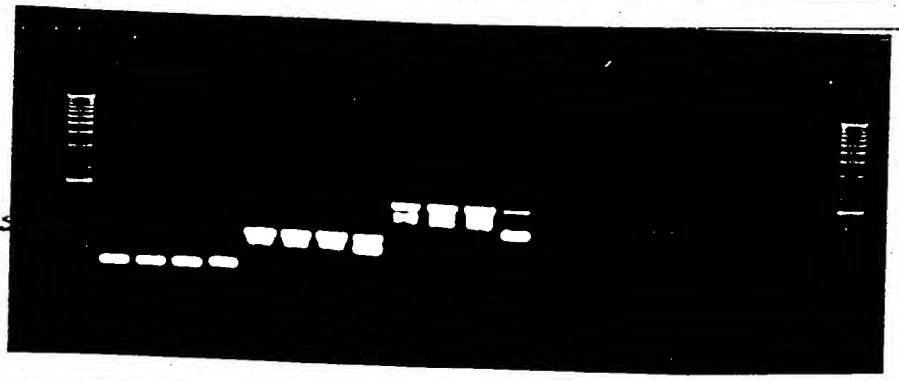
Pause the 9600's during later part of the 70°C, 2' xtension to withdraw 25ul samples at 25, 30, 35 cycles - +2.8ul Blue Juice

d & Understood by m ,	Date	Invented by	Date
Polan	7/7/95	Recorded by Carolyn Combs	7/6/95

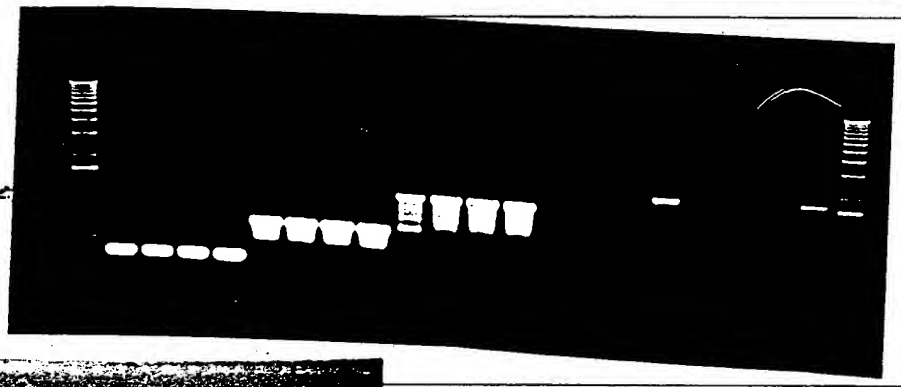
primer annealing temp: 6681 53° 55° 57° 59° | 7069 53° 55° 57° 59° | 407 | 1u Taq 806 | 2u Taq 806



25 cycles



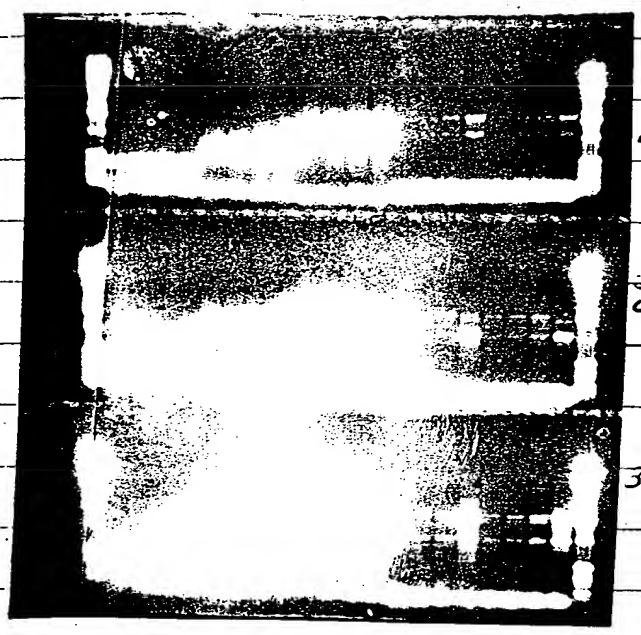
30 cycles



35 cycles

Note: 59°
cycles - I
9600 during
to 94°C so
product is,
ssDNA-acc
for the 2.
in 7069 & 4

Carolyn Combs
7/6/95



25 cycles

30 cycles

35 cycles

- 59°C annealing temp for 25 cycles gave a good produc yield and the least nonspecifi products for primers 6681, 7069, 407
- Try higher annealing temps fa primer 806 because product i beginning to come up at 59°C

Witnessed & Understood by me,	Date	Invented by	Date
BPolanyk	7/7/95	Recorded by Carolyn Combs	7/6/95

m13 PCR system: titration of [Tne] and [Taq] with 6681, 7069, & 407 primers - 3 cycle #s

g N

rpose: 1) To optimize the [Taq] -> 1u/100ul rxn was previously found to be inhibitory, so lower conc. will be tried - 0.25 u/rx.

2) To see if Tne can synthesize any of the products expected w/ 6681, 7069, &/or 407 primers + anchor primer - no product was made (p.44) when 5u Tne/100ul rxn was tried. The [template] and [primers] that were optimal for Taq will be used in the Tne PCR rxns. .1 - 1 u will be tried

- 25, 30, 35 cycle samples will be taken
- 59°C annealing temperature, 400nM primers, 100pg/100ul rxn m13 target

terials:

mix [A] w/ 6681, for 11.5 rxns

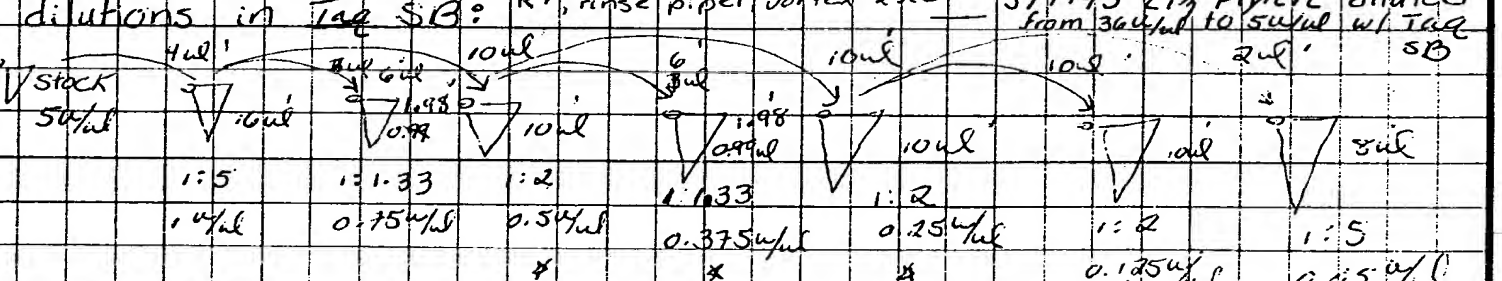
885.5 ul	H_2O	✓
115 ul	10x PCR buffer	✓
34.5 ul	50mM $MgCl_2$	Cf = 1.5mM ✓
23 ul	10mM dNTPs	Cf = 200uM ✓
23 ul	20uM anchor ✓	Cf = 400nM each
23 ul	20uM 6681 primer ✓	
23 ul	M13RF 50 pg/ul ✓	Cf = 100pg/rxn
1127 ul		

(see p. 69 for dilution of stock to 50 pg/ul for FAS 701)

mix [B] same as [A] but use 23ul, 20uM 7069 primer ✓

mix [C] same as [A] but use 23ul, 20uM 407 primer ✓

de dilutions in Taq SB: RT, rinse pipet, vortex 2sec — 5/7/95 Liz Flynn diluted from 3u/ul to 5u/ul w/ Taq SB



lso do same dilutions w/ Taq in Taq SB - only # dil will be used

op soln = 100mM EDTA → 80ul 0.5M EDTA + needed to kill exo
5x Blue Juice + 320ul 10x Blue Juice ice won't kill it
400ul

To Page No.

d & Understood by me,	Date	Invented by	Date
Polano	7/7/95	Recorded by Carlos (Lorie)	7/6/95

Project No._____

76 Book No._____ TITLE _____

From Page No.__

rxn # 1 2 3 4 5 6 7 8 → 14 15 - 21

98ul [A] 98ul [B] 98ul [C]

1 ½ul Tne 2ul

0.75 ½l Tne 2ul

0.5 ½l Tne 2ul

0.375 ½l Tne 2ul

0.25 ½l Tne 2ul

0.125 ½l Tne 2ul

0.05 ½l Tne 2ul

100ul

same as 1-7

same 1-

*remove 25ul rxn after 25, 30, 35 cycles to 2.8ul Blue Juice

note: 59°C annealing temp, 10:40 am - 2

94°C
94°C, 15"
59°C, 30"
70°C 2' extension
4°C final

> Lab 15 program 74 9600

rxn # 22 23 24 25 26 - 29 30 - 33

98ul [A] 98ul [B] 98ul [C]

0.5 ½l Taq 2ul

0.375 ½l Taq 2ul

0.25 ½l Taq 2ul

0.125 ½l Taq 2ul

0.05 ½l Taq

2 2 2 2

2 2 2 2

30 cycles only

** remove 25ul to 2.8ul Blue Juice

* remove 25ul of Tne rxns after 25, 30, 35 cycles during last part of 2min, 70°C elongation into 4ul stop soln in microtiter plate. The final [EDTA] = 10mM
[Blue Juice] = 1.1X

** remove 25ul of Taq rxns after 30 cycles + 4ul stop soln

To Pag N

Witnessed & Understood by m , Molano

Date 7/7/95

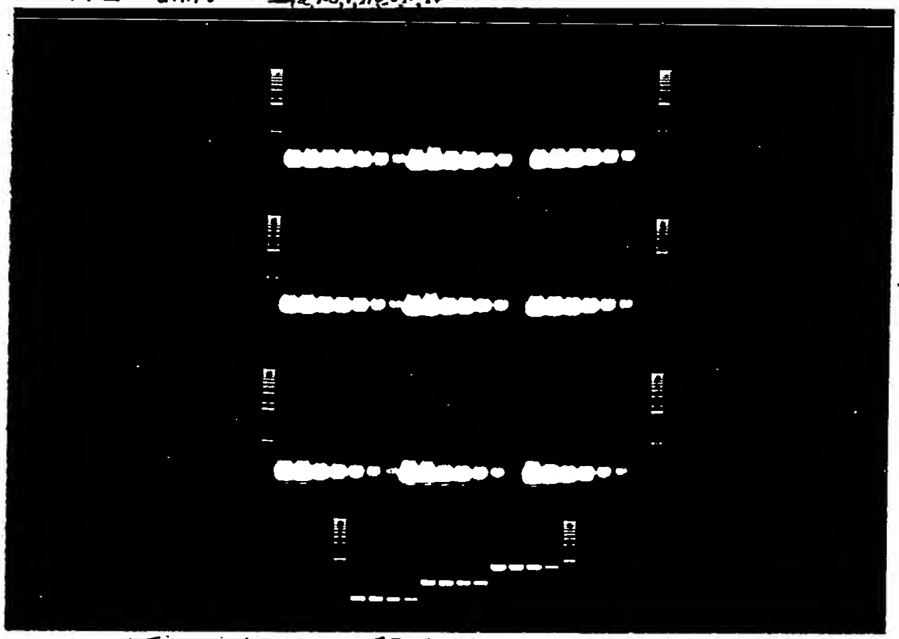
Invent d by R Gardner

Date 7/7/95 7/6/95

R c rd d by Carolyn Coombs

'ag N ___ Results

ected product size	380 ~~308~~ bp	768bp	1,356 bp
primer #	6681	7064	407
Tne units	12,1.5,1,.75,.5,.25	same	same

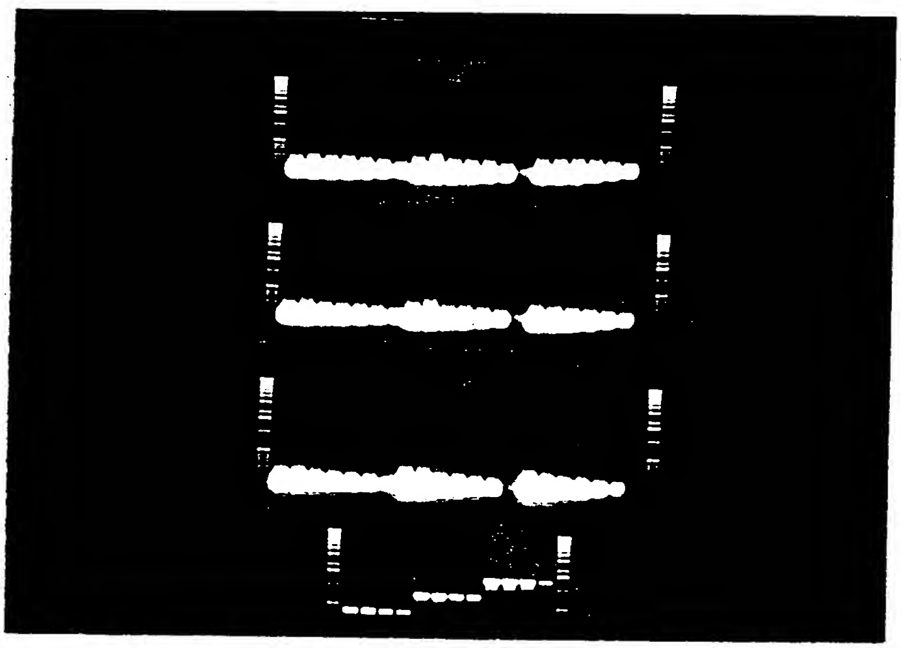


← 35 cycles
← 30 cycles
← 25 cycles
} Tne
← Taq 30 cycles

Taq units 1,.75,.5,.25

1) Taq made the expected specific products. 1u/100ul rxn Taq was optimal

2) Tne didn't make any specific products - only primer-dimers
more Tne gave more primer dimers
- try primer extens exp - low pol rate?



longer exposure of same gel

ed & Understo d by me, [signature] | Dat 7/13/95 | Invented by | Date 7/7/95
Recorded by [signature]

PCR with old & new Taq

Project No.

78 Book No. TITLE GAPDH pUC

Exhibit 127
Appl. No. 09/558,421

From Pag No.

	A	B	
5 x Chem (w. dNTPs) (P2, 10)	100 ✓	100 ✓	10 R
10 mM dNTPs	10 ✓	10 ✓	(200 µM
Human placen genomic DNA (HS #2 19/4) 80 ng/µl	12.5 ✓		(100 ng/5
GAPDH(+) 2112, 10µM	20 ✓		(400 nM Cf
GAPDH(-) 2113 10µM	20 ✓		
Mg OAc 100mM	—	2.5 ✓	(Cf Mg for Cf=1. for B
pUC DNA xmnI 25 pg/µl		10 ✓	
2836, 10µM		20 ✓	
2837, 10µM		20 ✓	
H_2O	317.5 ✓	317.5 ✓	
	480	480	

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

A 48 →

B 48 →

The 5-7-95 (Liz)

0.5		2						2									
1			2						2								
1.5				2		2	2.5				2	2.5					
2																	

The 55°C/µl Adam 3-2-95

dilute 2x/µl					1	2						1	2				
Taq 2 u/µl						1	2						1	2			

all dilutions made with Taq storage buffer

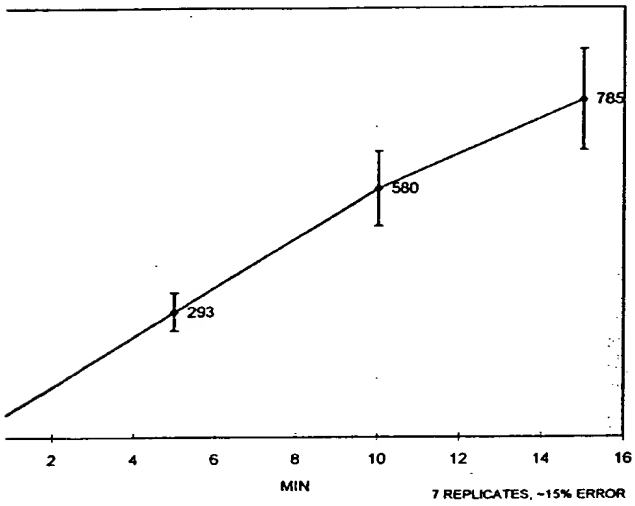
T Page N

Witness d & Understo d by m ,	Dat	Inv nted by	Dat
	7/14/95	R cord d by	7-10-95

g N .__

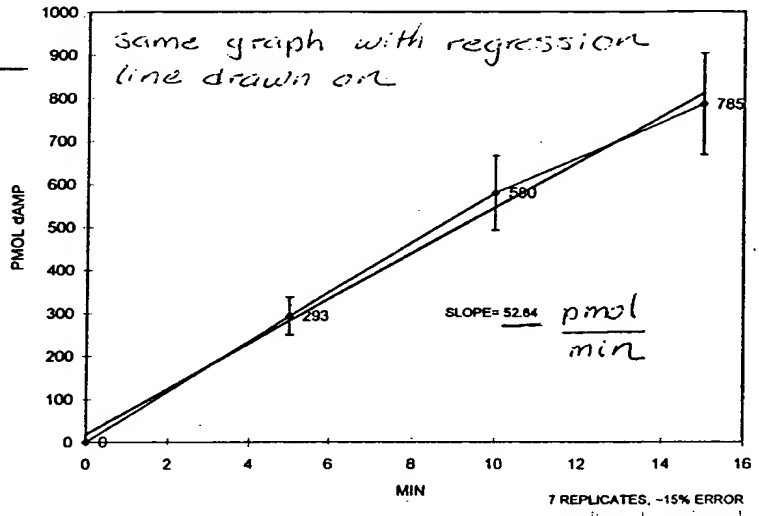
Sheet3 Chart 1

'S Tfl/Vent IN EPICENTER'S SB: TURNOVER AT TIME ZERO IN STABILITY STUDY (6/28/95)



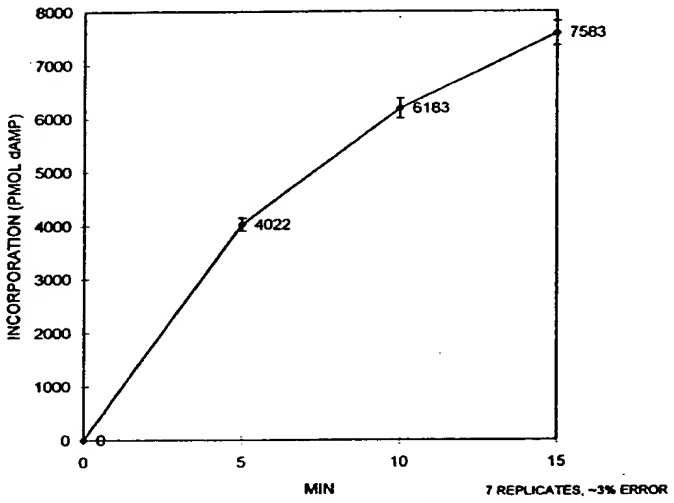
Sheet3 Chart 1

LTI'S Tfl/Vent IN EPICENTER'S SB: TURNOVER AT TIME ZERO IN STABILITY STUDY (6/28/95)



Sheet4 Chart 1

LTI'S Tfl/Vent: POLYMERIZATION AT ZERO TIME POINT IN STABILITY STUDY (6/28/95)



calculations:

To Pag No.____

ed & Und rstood by me,	Dat	Invented by	Date 7/11/95
		Rec rded by Carolyn Combs	

M13 PCR: Titration of Tne & Taq in (Cheng) buffer

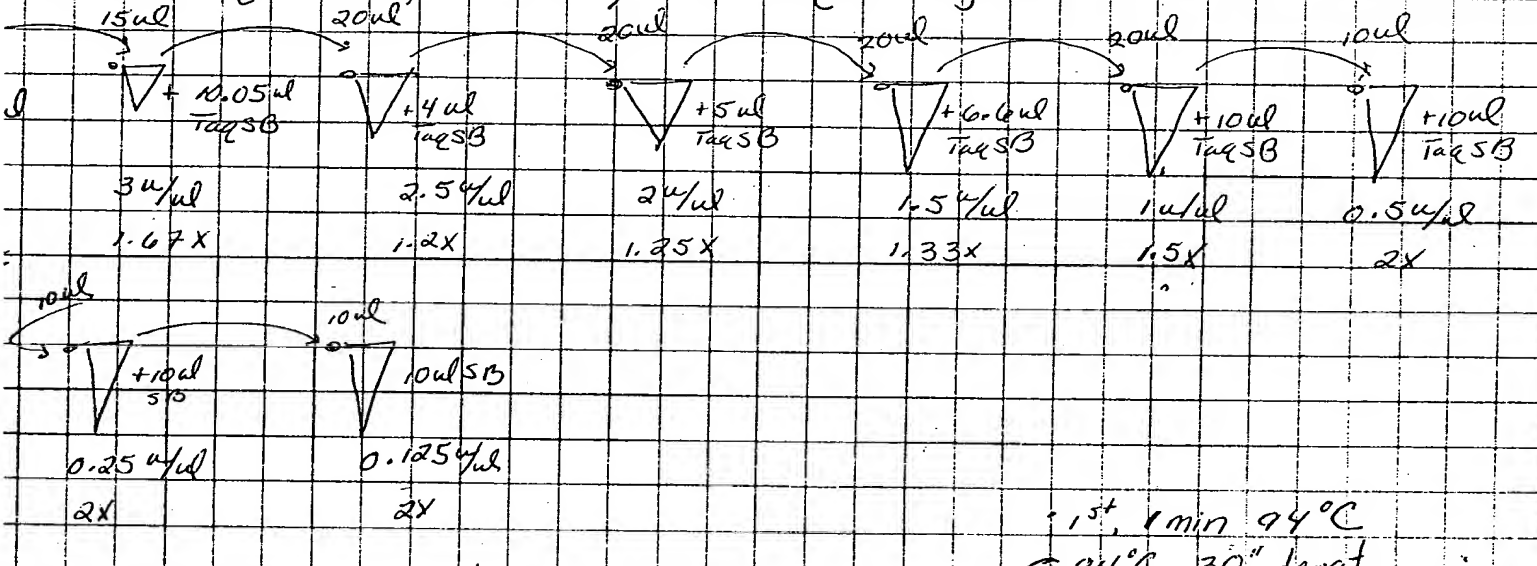
Page No. _____

purpose: Can we make the 380bp M13 PCR product w/ Tne? Earlier p. 77 w Tne failed to make this product in 10x PCR buffer.

4 for ~~17~~ 18 rxns, 100ul per rxn: 340 300ul 5x Cheng buffer
34 30ul 10mM dNTPs C_f = 200uM
34 30ul 50 pg/ul M13mp19 RF* in TC C_f = 100fM
34 30ul 20uM anchor primer
34 30ul 20uM 6681 primer C_f = 400nM
1190 ul H_2O

1666ul +470ul

ions of Taq & Tne (5/7/95 Liz) in Taq storage buffer (SB)



1 2 3 4 5 6 7 8 | 9 10 11 12 13 14 15 16

98ul A ——→

2ul +Taq

2ul Tne (5-7-95 Liz)

2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2

100ul

1st, 1min 94°C
cycle: 94°C 30" denat.
55°C annealing temp 30"
72°C exten 2'
remove ~~25~~ 35ul aliquots after 25, 30, 35 cycles
program 76
1pm — 4pm
16 x 3 = 48

*remove 25ul p. 79
+ 3ul STOP soln (w EDTA)
*run 25ul

To Page No. _____

d & Understood by me,
Roland

Date 7/14/95

Invented by

Recorded by Carolyn Ewing

Date 7/11/95

Project No.

84 Book No. TITLE m13 PCR: 3 small products w/ Tne

From Page No.

purpose: To determine if Tne can make the 3 smallest m13 PCR produ
380 bp, 768, 1356 bp, ~~380~~ using the conditions whic
worked for gapDH & pUC p.80 { Cheng buffer, 400nM p
35 cycles { 55°C annealing temp
94°C 30" denat, 100 pg/r
72°C 2' extention

If the products are made, we can use these conditions for
~~materi~~ m13 primer extention experiments

materials: mix for 15, 50ul rxns

[A] 150ul 5x Cheng
510ul H_2O
15ul 10mM dNTPs
15ul 50pg/ul m13mp19 RF
15ul 20uM anchor primer
705ul

[B] = 235ul A + 15ul 20uM 6681
[C] = 235ul A + 15ul 20uM 7069
[D] = 235ul A + 15ul 20uM 407

dilutions of Tne (5/17/95 Liz) in Taq storage buffer:

5u/ul stock → 10ul → +28.3ul TaqSB 33.3ul → 1:3.33x, 1.5u/ul → 20ul → +10ul TaqSB 30ul → 1:1.5, 1u/ul → 20ul → +20ul TaqSB 40ul → 1:2, 0.5u/ul → 20ul → +20ul TaqSB → 1:2, 0.25u/ul → 20ul → +20ul TaqSB → 1:2, 0.12

rxn	1	2	3	4	5	6	7	8	9	10	11	12	13
[B]	48	48	48ul										
[C]				48ul →									
[D]									48ul →				
1.5u/ul Tne				2					2				
1u/ul	2				2					2			
0.5u/ul		2				2					2		
0.25u/ul			2				2					2	
0.125u/ul								2					2

→ start rxns on ice w/ an

Result on p.86
Only the 380k product was made.

T Page N

Witn ss d & Understood by me, DDolan

Date 7/14/95

Invent d by

Rec rded by Dawson Pimb

Dat 7/12/95

same experiment as P. 64 except ^{32}P 23 instead of ^{32}P dA
increasing T/O with time (P 64-68) due to strong pause sites
visible on an agarose gel ?

5 ng/λ 23mer — 35 μl — 175 ng
5x kinase buffer — 10.2 μl — 23 pmol 23mer
PNK — 1.2 μl
γ ATP 10 mCi/ml 7-14-95 — 5

51.4 μl

37°C, 30 min, → 55°C, 5 min

51.4 μl
H_2O — 42.6
M13 mp19 0.2 μg/λ — 106 — 11.5 pmol circle tot
0.79 nmol nt/λ
.109 pmol circle/λ

200

23mer / circle = 2

70°C, 5' cool slow

f = 0.41 nmol nt M13/μl

it will be ~23.7 total nmol nt/100 μl Rxn
same as for P. 64

To Page No.______

d & Understood by me, Polans — Date 7/14/95 — Invented by [signature] — Date 7-13-95 — Recorded by — RJ

[redacted]

Project No.________

Book No.________ TITLE ________________________

From Pag No.__

(A) 3.5 Rxns

^{32}P 23 mer - mp19 (P87) 171.1
0.41 nmol nt/μl
.0566 pmol circle/λ
5X Cheng (no dNTPs) 70
P21, 10

10 mM dNTPs 1.75 Cf = 50 μM
H_2O 100.15

Vf = 343 (use 98 μl/100 μl R:

	①	②	③
[A]	98 μl	⟶	
Tfl/Vent (epicenter Tfl) 5-16-95	2		
Tfl lot 31010A-502 1 u/λ (epicenter units)		1.88 μl	
Vent 2 u/μl lot #17 opened 2-24-95			2

(1.88 u total in ← its only 0.94 (epicenter units) due with Vent by Nir

(1.88 unit Tfl

(4 units) ... more Vent than in Tfl, in order to get full length products)

68°C. Remove 8 μl at 1, 2, 5, 10, 15, 20, 40, 60, 9 to 1 μl 10X 'blue juice' mM E.

run on agarose (same as P 56, 9)

To Pag N

Witnessed & Understood by me, DPolamp Dat 7/14/95

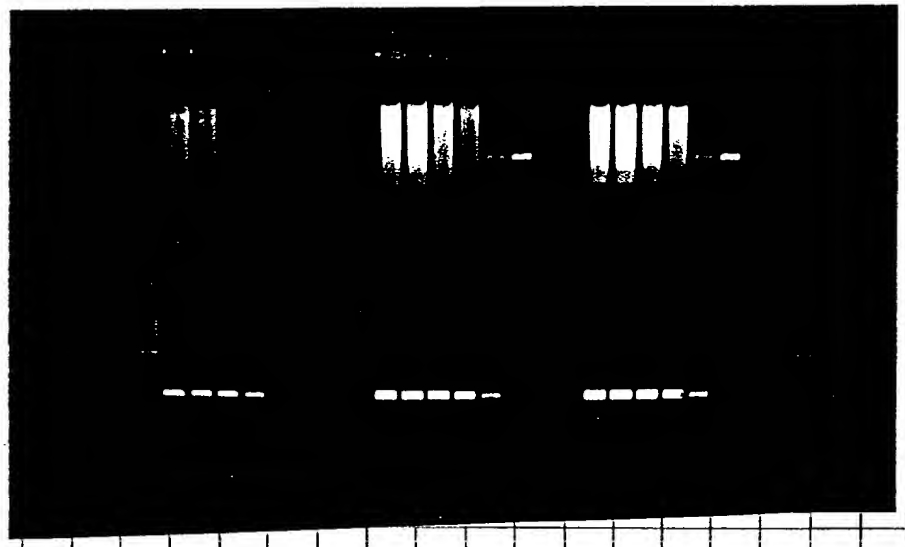
Inv nted by [signature] Dat 7-14-95

R c rd d by

1% agarose gel

top empty

9-16	9-16	9-16	Tne 9-16 decreasing enzy
25	30	35	
1-8	1-8	1-8	Taq 1-8 decreasing enzy - run into gel 1st
35	30	35	Tne from p. 80



same photo as p. taken from a furth distance to capt the ~~with~~ bottom of gel

To Pag N.

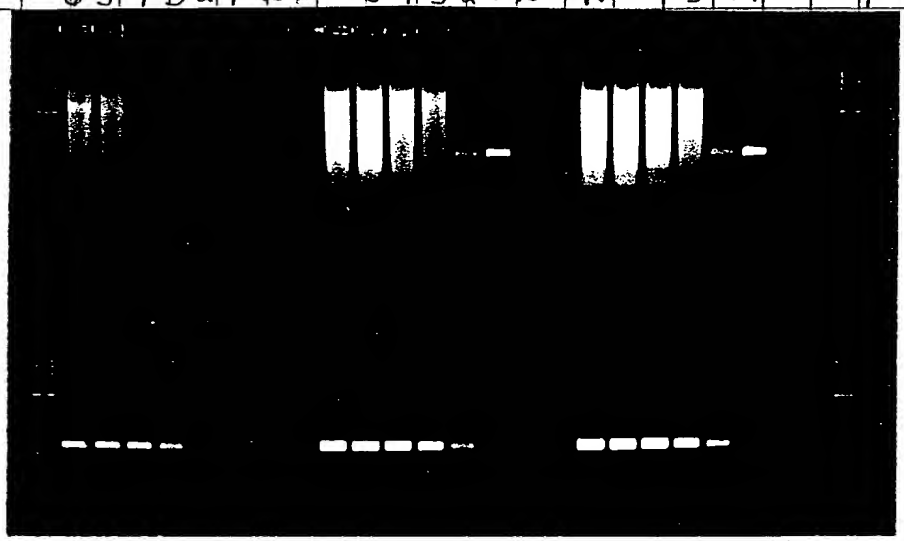
tnessed & Understood by m ,	Date	Inv nted by	Date
7/14/95		R c rded by	7/14/95

Page No.__

?heng buffer, 380bp product expected

25 cycles | 30 cycles | 35 cycles

units 6 5 4 3 2 1 .5 .25 | 6 5 4 3 2 1 .5 .25 | 6 5 4 3 2 1 .5 .25 | 1/pool rxns



Tne

rTaq

- 1 unit Tne is optimal, 35 cycles ~~2~~ 1.5
- 6 units rTaq gave the most product for all cycle #'s

To Page No.____

ed & Understood by me,
Polcyn

Date 7/14/95

Invented by

Recorded by Cindy Combs

Dat 7/14/95

213 PCR: Δ PCR buffer into Cheng buffer

Proj No.

purpose: To find out what component(s) of Cheng are important for making the broad smear (vs narrow, low mw smear made w/ PCR buffer)

1) 3g agarose.
6mL 50x TAE
294mL H_2O
300mL wt 514.59 w/ magnet
+ 20ul EtBr

a/s: [A] for 35 rxns = 35 ul 20uM anchor primer
35 ul 20uM 6681 primer
35 ul 10mM dNTPs
910 ul H_2O
1015

[B] w/ target
507.5 ul of [A]
+ 17.5 ul 50pg/ul m13 RF

[C] no target DNA
507.5 ul of [A]
+ 17.5 ul H_2O

*Vens 2/10/95

[D] = mix of Tricine 200mM + MgOAc 10.05 mM to add KOAc to = 10x ~~working~~ stock
200ul of 1M Tricine pH 9 (from Ven)
+ 10.05 ul of 1M MgOAC
+ 789.95 ul H_2O
1mL

[E] = mix of 166.6mM Tricine pH 9, 708 mM KOAc, 8.75mM mgOAC
is 8.33X stock
166.6 ul 1M Trine pH 9
354 ul 2M KOAC
8.75 ul 1M MgOAC
470.65 ul H_2O

Tne (5-7-95) Liz 5 ul diluted to 0.2 u/ul w/ Taq storage buffer + 6.2 ul Tne = 148.8 ul
τ 12/7/95

x:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 → 28
	30ul [B] target														30ul [C] no target
uffer	5														same as 15-28 without target
Cl_2	1.5														
		5	5	5											
Cl		10													
KOAc			2.35	5.88											
					6	6	6	6	6	6	6	6	6	6	
erol						2	5	8				8	8	8	
SO									.5	.75	1	.5	.75	1	
	8.5	0	7.65	4.12	9	7	4	1	8.5	8.25	8	0.5	.25	0	
ne	5														
	50ul														

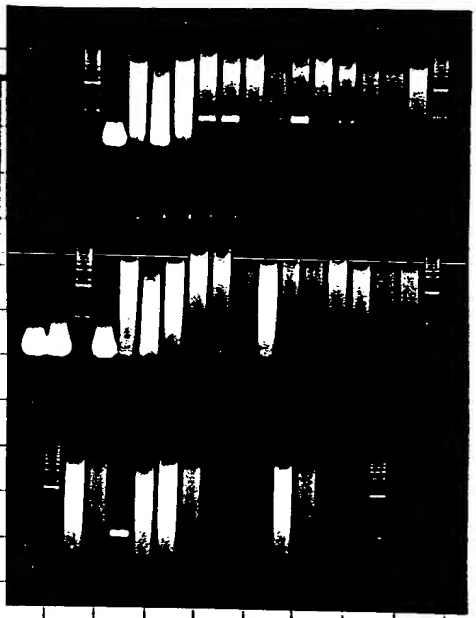
To Page No.

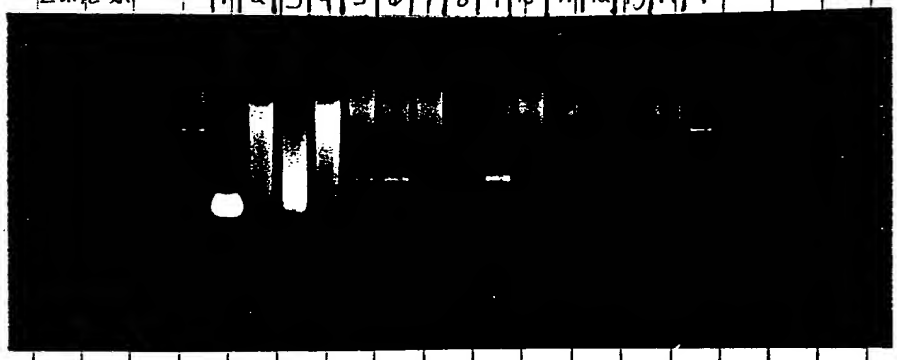
d & Understood by me,	Date	Invented by	Date
Polarp	7/14/95	Recorded by Carolyn Lamb	7/14/95

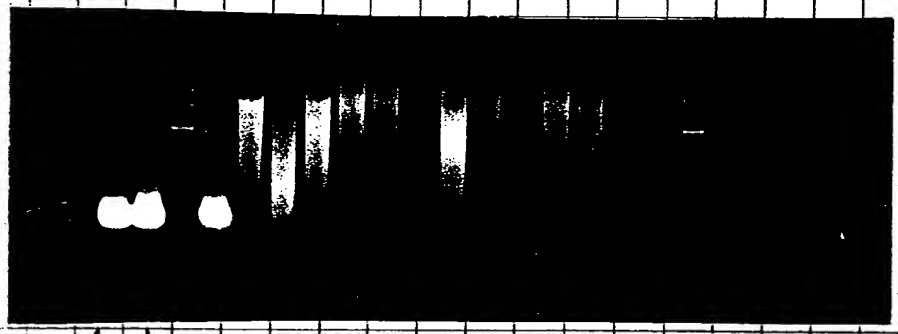
Project No._____
Book No._____ TITLE _____

From Page No.__

Buffer components	1x buffer	mM	chem
Tris pH 8.4	20		
KCl	50	50	
$MgCl_2$	1.5		
Tricine pH 9		20	
MgOAc		1.05	
KOAc		20 50 85	
glycerol		2.5 5 8	3 8 8 8 % ← really is % glycerol due to contribution from enzyme
DMSO		1 1.5 2	1 1.5 2 %
Lane ##		1 2 3 4 5 6 7 8 9 10 11 12 13 14	15 1Kb ladder







anchor primer alone (6681) primer alone

c. Combs 7/14/95

+m13 template + 2 primers for 380bp produc

* Note, the enzym brings glycerol i rxn, 50 [glycero 7 10, 13 %

c. Combs 7/14/95

−m13 template + 2 primers

no template, only

expt on p.8

1) The transition from "primer dimer" (smallest band) to smear occurred when Tricine pH 9 + MgOAc 1.05mM was substituted for Tris pH 8.4 + MgCl
2) Product was made when 85mM KOAc was added to the tricine MgOAc buffer - glycerol + DMSO were not required to make produc
3) Less glycerol + DMSO resulted in more product synthesis
4) The smears are present in the plus and minus target lanes
5) The "primer dimer" is made when either primer is present alone if both primers are present as long as PCR buffer is used

To Pag

Witn ssed & Understood by m , D Polans — Dat 7/14/95

Invent d by — Dat

Record d by Dustin Combs — 7/14/95

Project No._______

Book No._______ TITLE is ^{32}P primer incorporated into either the "primer dimer" or smear see Tne on P. 86 ?

From Page N .__

	(– Target) A	(+ Target) B	(85 mM KOAc) C	10 Rxns
1 M Tris pH 8.4	10			20 mM
3 M KCl	8.33	8.33	–	(50 mM)
50 mM MgCl2	15			(1.5 mM)
1 M Tricine pH 9		10	10	20 mM
MgOAc 50 mM		10.5	10.5	(1.05 mM)
M13 RF 50 pg/µl	–	10	10	(50 pg/50λ PCR
10 mM dNTPs	10	10	10	
			21.25 ←	2M KOAc
H2O	356.7	361.2	~~350~~ 547.25	
Vf =	410	410	410	

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	41 µl →							→									
B / C									4 µl →								→ 41
6681 10 µM	2		2		2		2		2		2		2		2		2
6681 10 µM				2				2				2				2	
301 10 µM		2		2		2		2		2		2		2		2	
c6301 10 µM			2				2				2				2		2

Cf = 40 nM

Tne 5-79(2) 5 µl → 2
0.2 u/µl diluted in Taq SB

note for 3 µl 8% PAGE its 0.08 µl ^{32}P primer

35 cycles as per P 86

mix 20 µl PCR with 2 µl BJ, 100 mM EDTA, load 10 µl on 0.8% Ag as per P56, 9 190 V start ~2 PM (?)
Movies as per AK 6 of 7 test 4:30

To remaining 30 µl PCR add 15 µl cycle seq stop, load 3 µl on 8% PAGE (wells 1-17 are PCR reactions

for sequence Rxn use ^{32}P 6681 • mp19 ss DNA (PT1) as per (P27, 4)

To Pag N

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	7/14/95	[signature] Recorded by	7-14-95

^{32}P 6681: and ^{32}P 6301 anchor

Project No. ______ Book No. ______

No.__ (cam see P150, 9)

	①	②		
100 μM 6301 (anchor)	1.57		✓	Cf = 10 μM Cf
100 μM 6681		1.57	✓	
^{32}P γ ATP pmCi/μl Cf = 7-14-95	10	10	✓	
PNK 1 u/λ	1	1	✓	Cf at 1X kinase buffer
5X Kinase buffer	3.1	3.1	✓	35 mM Tris pH7.6
	15.66 μl	15.6		50 mM KCl; 5 mM $MgCl_2$
μM 6301 cold	78.3			
μM 6681 cold		78.3		
	93.98			

e dilute hot primer with 5 parts cold primer
∴ contribution to PCR of p90 is:

	primer	in PCR
$MgCl_2$	0.83	0.033
Tris pH7.6	5.8 mM	0.23 mM
KCl	8.3	0.33

^{32}P 6681 • mp19 for sequence

mp19	0.26 μg/μl 0.109 pmol circle/λ	10 μl	(1.09 pm circle
^{32}P 6681	10 μM primer	1	10 pm primer
		70°C	cool slow

for 3 μl on PAGE is ~0.04 μl primer

To Page No.____

Read & Understood by me, Polarp — Date 7/14/95

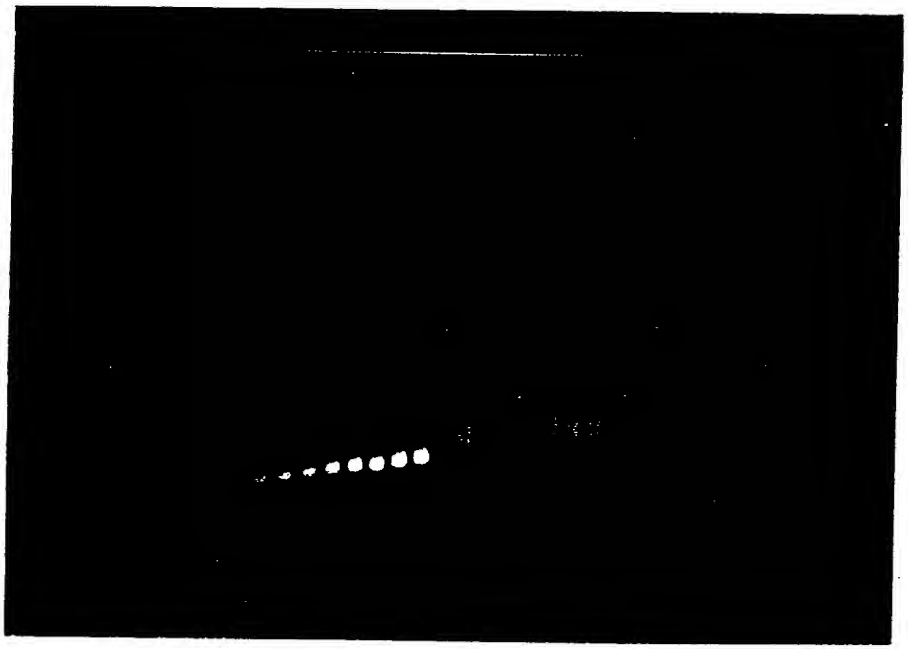
Invented by [signature] — Recorded by — Date 7-14-95

From Page

1.5 mM $MgCl_2$ 1.05 $MgOAc$

Tris pH 8.4 Tricine pH 9

KOAc 85 mM



PCR

Tne

^{32}P primers

→ 0.8 % Agarose → EtBr

→ Autorad

→ 8% PAGE (denaturing) → Autorad

Witn ssed & Underst od by m , | Dat | Inv nt d by | Dat 7-14-95

R c rded by

PAGE 93 OF NOTEBOOK WAS BLANK

Project No._____

94 Book No._____ TITLE Primer dimer/smear transition: effect of Tris 8.4/Tricine 9, [Mg], MgOAc vs MgCl

From Page No.___ 7/14/95 purpose: To determine which buffer component(s) were resp

1) For the transition seen from "primer dimer" to "smear" on p.86 14
The "primer-dimer" condition was 20mM Tris 8.4
50mM KCl
1.5mM $MgCl_2$
The "smear" condition was 20mM Tricine pH 9
50mM KCl
1.05mM MgOAc
what comp is causin transitio Tris 8.4 vs [Mg] MgOAc ?

2) Is the "smear" or "primer dimer" due to DNA contamination of the prep? do rxns w/o D

materials:

180.ul 0.2 u/ul Tne (5-7-95 Liq) → 7.2ul Tne stock 5u/ul
+ 172.8ul Taq storage buffer

! 100mM Tris 8.5 * note this is a change from the original 8.4 "
dimer" condition 100ul 1M Tris 8.5 (RL)
900ul H_2O

! 100mM Tricine pH 9 100ul 1M Tricine pH 9 (Nen 2/10/95)
900ul H_2O

~~5.25~~ ~~10.5~~ mM $MgCl_2$ ~~210~~ul 50mM $MgCl_2$ — 105ul
~~790~~ul H_2O — 895ul

! ~~7.5~~ ~~15~~ mM $MgCl_2$ ~~300~~ul 50mM $MgCl_2$ — 150ul
~~700~~ul H_2O — 850ul

5.25
~~10.5~~ mM MgOAc 10.5ul 1M MgOAc → 5.25 — 10.5ul 5.25
989.5 ul H_2O 1989.5ul 994.7
2mL

7.5
~~15~~ mM MgOAc 15ul 1M MgOAc — 7.5ul 7.5ul
485ul H_2O — 1985ul 992.5ul
2mL

T Pag N

Witn ss d & Und rst od by me, Dat 7/24/95 Inv nt d by Dat 7/14/95
Rec rd d by Carolyn Pomb

e N .__

25:

A = 360ul 10mM dNTPs ✓
29.88ul 3M KCl ✓
726.12ul H_2O (1st) ✓
792ul

[B] template + primers
374ul [A] ✓
17ul 20uM anchor primer ✓
17ul 20uM M6681 primer ✓
17ul 50 pg/ul M13RF ✓
425ul

use 25ul per 50ul rxn → this will give 400nM primers, 50 pg template, 50mM KCl, 200 uM dNTPs in 50ul rxns

[C] no DNA, only enzyme
374ul [A] ✓
51ul H_2O ✓
425ul

up each rxn in duplicate

r components:

rxn #	1,2	3,4	5,6	7,8	9,10	11,12	13,14	15,16
	small smear condition = rxn buffer	tricine instead of Tris	1.05 mg2+ instead of 1.5	MgOAc instead of $MgCl_2$	change (Mg2+ + anion)	broad smear condition	Tricine w/ high (Mg2+)	Tricine w/ $MgCl_2$
Tris pH 8.4	✓		✓	✓	✓			
KCl	✓	✓	✓	✓	✓	✓	✓	✓
$MgCl_2$	✓	✓						
Tricine pH 9		✓				✓	✓	✓
n $MgCl_2$			✓					✓
MgOAc				✓			✓	
m MgOAc					✓	✓		

To Page No._____

& Understood by me,	Date	Invented by	Date
Dulany	7/24/95		7/14/95
		Recorded by Carolyn Combs	

Project No.

96 Book No. TITLE

From Page No.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
100mM Tris 8.5	10	10			10	10	10	10	10	10							10	10														
100mM Tricine 9			10	10							10	10	10	10	10	10			10	10												
7.5 ~~5~~mM MgCl	10	10	10	10													10	10	10	10												
5.25 ~~4.25~~ mM MgCl					10	10									10	10					10	10										
7.5 ~~5~~mM MgOAc							10	10					10	10									10	10								
5.25 ~~4.25~~ mM MgOAc									10	10	10	10													10	10	10	10				
mix	[B] 25ul = template + primers + dNTPs + KCl																[C] 25ul = H_2O + dNTPs + KCl "No DNA"															
0.24ul Taq (5-7-95 Lig)	5ul brings in ~5% glycerol																															
50ul	add enz, vorter, quick spin (4°C) to start runs																															
	then cycle in Lab IS 9600 program 76																															
	1 min 94°C																															
	30 sec 94°C, 30 sec 55°C, 2 min 72°C — 35 cycles																															
	4°C																															
	4ul																															
	STOP crns w/ 4ul 10X STOP = 10mM EDTA p. 79																															
	1:10																															
	1:10																															
	Run 25ul on 1% agarose gel																															
	8x blue ice																															

T Pag

Witn ssed & Und rstood by m ,	Dat	Inv nt d by	Dat
	7/24/95		7/14/95
		R c rd d by Sandra Pombo	

g N ._

- Tris w/ 1.5mM $MgCl_2$
- Tricine w/ 1.5mM $MgCl_2$
- Tris w/ 1.05mM $MgCl_2$
- Tris w/ 1.5mM MgOAc
- Tris w/ 1.05mM magnetate
- Tricine w/ 1.05mM MgOAc
- Tricine w/ 1.5mM MgOAc
- Tricine w/ 1.05mM $MgCl_2$

template + primers

no DNA
ie no template
no primers

ecomb 5/14/95 ec

all at 50mM KCl

wer concentration of Mg^{2+} in Cheng vs PCR buffer is responsible for the broader smear. The Tris / tricine pH difference and $MgCl_2$ vs MgOAc do not affect the transition from small (narrow) to broad smear.

is model: distance between primers
low [Mg]
high [KOAc]
low [Tne]

short = small narrow smear

long = broad smear

✓ by decreasing primer annealing
✓ by inhibiting Tne from binding primers

To Page No._____

& Understood by me,	Date	Invented by	Date
clamp	7/21/95		7/14/95
		Recorded by Carolyn Comb	

e N. ___

15 9600 - 94°C 1min
94°C 30sec
55°C 30sec } 35 cycles
72°C 2min
4°C hold

rxns by adding 7.5 ul of Stop soln = 2x Blue Juice Cf = 1x
100mM EDTA Cf = 12.5mM EDTA

Stop soln made for future use = 50% glycerol
100 mM EDTA (10x)
0.6x TAE
BØB

for 5mL - 2.5ml 100% glycerol
1mL 0.5M EDTA
1.5mL 1x TAE = 30ul 50x TAE stock + 1470 ul H_2O
+ pinch of BØB

To Page No. ___

& Understood by m ,	Date	Invent d by	Date
Polaiyp	7/14/95	Recorded by Cmb	7/15/95

Project No.
100 Book No. TITLE Buffers

From Page No.

Buffer C, 1L ref NB 9, pg 162

17.1 mL 1m K phos monobasic
7.9 mL 1m K phos dibasic
50 mL glycerol
149.12 g KCl Cf = 2M
0.2 mL 0.5m EDTA
350 ul 14.3 m BME - add
qs to 1L w/ H_2O

1m K phos monobasic
68.045 g
qs to 500 mL

1m K phos dibasic
114.115 g
qs to 500 mL

+ 1 mL of 50% Tween 20 + NP40 - post filter det 1m/L + B-ME

Buffer D, 8L ref. NB 9 p 182

200 mL 1m Tris 7.5
1.6 mL 0.5m EDTA
640 mL glycerol
2.8 mL 14.3 m BME + add
29.8 g KCl Cf = 50mM
qs to 8L

make 0.01% NP40 + Tween 20 for dialysis → 2L D + 700 ul 400 ul 5.0% N
& 0.05% for Heparin column - filter 1L + add 1mL 5.
NP40 + Tween 20 + 350 ul.

Buffer C, 500mL ref. NB 9 p 182

det 0.05% final 1mL/L

12.5 mL 1m Tris 7.5
0.1 mL 0.5m EDTA
40 mL glycerol add
0.175 mL B-me
74.5 g KCl Cf = 2M
qs to 500mL

+ 0.5mL 50% Tween 20 + NP40 after filtering

T Pag N

Witness d & Understood by me, Date 7/24/95 Inv nt d by Dat
R c rd d by 7.18.95

No. 7/18/95

ml Taq extract buffer (P.160, 3) with 0.05% each NP40 / Tween 20
50ul 10mM PMSF
7.6ul 143M βME
25ul Tween 20 > 50% mix of both → 5mL each
25ul NP40

g cells -70°C Thorton shelf, Lab 16 -chip off 9503-15-764-01-001K
Tne 5 Pol
ml Taq extract buffer mix w/ spat in 50mL
0.2g cells/mL + 10mL pipet Falcon

ake tune - on xL2020 → { to tune, turn small to min
then min 1-5 minimize, not over 70
stop

9x 30sec pulses in ice-H_2O bath, ~1min between pulses - should turn browner

75°C in Falcon - H_2O bath, then cool in ice-H_2O

e Cf 200mM NaCl + 5% PEI to Cf = 0.5% → 16mL vol extract in ~~grad~~ cylinder
58.449/m 30mL centrifuge tube

$.2\ \frac{mole}{L} \times .016\ L = 0.0032\ m$

$\frac{x\ g}{58.449/m} = 0.0032\ m$

0.187g NaCl ✓

~~1.05mL~~ 1.08mL ✓

$\frac{.5\%\ 16mL}{5\%}$ ~~16mL~~ of 5% PEI while extract is stirring in c tube, 1 drop/sec

15min, 4°C
15min, 15K 5534, 4°C (~2700xg) DNA, cell debris & heat-denatured proteins will ppt.

ant into 25mL grad cylinder:
= FI' fraction 13.25mL of FI' - .2mL = 13.05mL
d AmSO4 to help it go into soln ⇒ 2.297g ammon. sul

To Page No.

d & Understood by me,	Date	Invented by	Date
[signature]	7/24/95	[signature]	7-18-95
		Recorded by Carolyn Combs	

Project No._____

102 Book No._____ TITLE _____

From Page No.___

remove 200ul aliquots of each fraction - eppendorf on
add Am SO4, ~~spin~~ stir 15 min, spin down 15K. 15', remove aliquot
remeasure volume

~~S30~~	0% SOA Am SO4	vol (mL)	g Am SO4 slowly, stir 15'
Fr I'	0	13.05	2.297g
S 1	30%	13.8	0.414g
S 2	35	13.5	0.1829 wrong 0.223g more added
S 3	~~40~~ 40	~~13.5~~ 13.75	~~0.405g 0.223g~~ 0.426g aliquot had
S 4	45	13.5	0.419
S 5	50	13.5	0.432
S 6	55	13.4	0.442
S 7	60	13.2	
S 8	65		
S 9	70		

mix: A { 150 ul 0.5 M Tapes 9.3 ×2 = 300ul 0.5 M Taps 9.3
6 ul 1M MgCl2 12 ul 1M MgCl2
50 ul 3M KCl 100 ul 3M KCl

made 2
~~4 mL~~ 6.4 mL

3 mL
200 ul mix A 412 ul mix A
6.3 ul ^{32}P dCTP ~~@~~ 10mCi/mL rec'd 7/14/95 - 12.6 ul ^{32}P dCTPα
60 ul 10mM dNTPs 120 ul 10mM dNTPs
405 ul 3.7 mg/mL gap activated DNA 810 ul 3.7 mg/mL activa
2.523 mL H_2O 5.046 mL H_2O
~~3.2 mL~~ for 66 rxns worth, use 48 ul/rxn 6.4 mL

1:100 in Taq dilution buffer 2ul aliquot + 198ul Taq dil bu
2ul dil + 48ul reaction mix
10', 74°C 50ul rxn
stop w/ 10ul 0.5M EDTA + 10 ul stop
spot 20ul on GFC filters spot 20ul

T Pag N

Witnessed & Understo d by m ,	Dat	Inv nted by	Dat
	7/24/95	R c rd d by	7.27.95

g N .__

$\left(\frac{cpm}{specific\ activity}\right)\left(\frac{40\,ul}{20\,ul}\right) = pmole$

			pmol	units/ul
1		CPM1		
7195 C. Combs				
1	no enz	71.00	7.55	
2	FI'	4679.00	498	7.47
3	s1	5411.00	576	
4	s2	5860.00	623	
5	s3	5434.00	578	
6	s4	3558.00	377	
7	s5	1394.00	148	
8	s6	299.00	31.8	→ 55% ammonium sulfate
9	mix	45046.00	2ul	
10	mix	44957.00	2ul	$\bar{x}$ = 45048
11	mix	45141.00	2ul	

specific activity = $\left(\frac{45048\,cpm}{20{,}000\ (4)\ pmole\ nt}\right)\left(\frac{50\,ul\,rxn}{2\,ul}\right) = 28.2\ \frac{cpm}{pmole\ nt}$

~~20,000~~ ~~5000~~ (4) pmole nt
10,000 pmole dCTP

$\frac{498\ pmole}{10\ nmole} \times 3$ (for 10') $= \frac{units}{2}(200) = 7.47\ \frac{units}{ul}$ in FI'

100,000 units / 3.5 g cells = 28,000 u/g

Liz - 22,000 u/g

To Page N .______

Read & Understood by me,	Date	Invented by	Date
[signature]	7/24/95	[signature]	7.18.95
		Recorded by [signature: Carolyn Combs]	

PAGE 104 OF NOTEBOOK WAS BLANK

Large scale (31.5 g cells)

Tne prep (continued P176, 9)

N — except use detergent in Tag ext buffer

31.5 g — 9503-15-714 01-001R Tne 5 pvl

326 ml — Tag ext buffer (RT PIPES, 3 : with 0.05% each Tween 20 / NP40

(Cf ~ 0.2 g/ml cells

stir cells in RT buffer in beaker

strain thru cheesecloth

Minigaulin

10,000 PSI ✓ — 1 pass

½ mL = FRI Tne, spun down cells 15 min 4°C - sup = FRI Tne

75°C, 15 min (total time after Temp reaches 75°C)

cool 1' fast in ice ~~slurry~~ Slurry

vol = 405 ml

NaCl added to Cf 200 mM

= 4.73 g ✓

200 mmole/L × 0.405 L = 81 mmole = 0.081 mole

x g / 58.449/m = .081 m

PEI for Cf = 0.5% add

45 ml 5% PEI pH 7.4 ✓ ~~add~~

add dropwise, stir 15' more ✓

spin GSA 13,000 RPM, 30 min ✓ Supe = FrI'/PEI

ammonium + divide in 2 / 2 bottles

To Page No.

I & Understood by me,	Date	Invented by	Dat
Steve	7 July 94	Recorded by	7-18-95

From Page No.__

vol of FRI'/PEI sup = 355 mL
removed 1/2 ml = FrI'/PEI

$\frac{351g}{1000mL} = \frac{124.69}{355mL}$ + ref R. Scopes. Protein Purification p. 304

add 124.605 g ammonium sulfate (ground up) for 55% sat
added slowly over ~ 15 min while stirring at 4°

stirred 15 min, 4°C

spun down pellet 13K, 30 min GSA RC-5B, 4°C → pellet contains DNA polyme
(spin in two bottles to produce 2 pellets of equal size)
saved sup at 4°C in case activity didn't come down
also 1/2 ml aliquot
respun 5K 5 min to pull as much liquid off the pellets as po
put the 2 pellets at -70°C

FPLC method for S200 column

method 5 bank 2

0	conc % B	0.0
0	conc % B	0.0
0	mL/min	1.5
0	port set	6.1
0	port set	6.1
0	valve pos	1.1
0	valve pos	2.1
400	conc % B	0.0
400	mL/min	1.5

METHOD 5 BANK 2

0.00 CONC %B 0
0.00 CONC %B 0
0.00 FLOWRATE 1.
0.00 PORT SET 6
0.00 PORT SET 6
0.00 VALVE POS 1
0.00 VALVE POS 2
CONC %B 0
FLOWRATE 1.

To Pag

Witnessed & Understood by me, Date 7/24/95 Invented by [signature] Dat 7-19-95
Recorded by [signature]

Page No. – wash with H_2O, (Hi Trap Pharmacia)
equilibrate a 10 ml blue column (used once and stored in 20% EtOH P191,9)
at 3 col vol/hr = 0.5 ml/min
for 2½ hrs (= 7 col vol) with buffer B

wash S200 with H_2O (180 ml = 1 col vol P178,9)
Equilibrate with buffer B
at ½ col vol/hr
= 90 ml/hr
= 1.5 ml/min } for S200 col will equilibrate O/N at 0.6 ml/min (= 3 col vol) in 500 ml in 14 hr

S200 column

Resuspend one of the two $AmSO_4$ pellets of P106 (= 0.5 of the total material from 1.5 g cells on P105) in buffer B (~~and~~ containing detergent) (as per P178, 9)
final vol = 0.89 ml spun out insoluble material in microfuge 15'
remove ~20 µl to assay later $AmSO_4$ resuspend

Load by gravity on 180 ml S200.
elute at ½ col vol/hr (ie. 1.5 ml/min) in buffer B
spun = 2A, 2 mm/min, 1.5 ml/min, 3 ml/frn 2 min/frn

To Page No.

d & Understood by me,	Date	Invented by	Date
D Calvo	7/24/91	Recorded by Carolyn Comb	7-19-91 7-20-91

From Page No.__

unit assay on fractions 3-17 from S200 column
2ul fraction + 61.25ul Taq dil buffer ⇒ 1:31.62
↳ 2ul + 61.25ul Taq dil buffer ⇒ 1:31.62 total of 1:1000 di
↳ 2ul of 1/1000 d:l + 48ul mix A p.102
& a no enz control
10min 74°C - temp varied between 72-77°C
10ul STOP 0.5M EDTA
20ul on GFC - wash 1x 10%, 3x 5% TCA, 2x EtOH
dry 20min, count

$\frac{\text{pmol}}{}$ ($\frac{\text{cpm}}{\text{S.A}}$)($\frac{60\text{ul}}{20\text{ul}}$) specific activity = 28.2 cpm/pmol

	SAM	CPM1		unit
	1 no enz	41.00	4.36	5% were cloudy — no enz
Fraction	2 3	52.00	5.63	3
&	3 4	51.00	5.43	4
from	4 5	45.00	4.79	5
S200	5 6	153.00	16.3	6
	6 7	721.00	76.7	7 34.5
	7 8	634.00	67.4	8 30.
	8 9	2027.00	216	9 97.
	9 10	4597.00	489	10 22C
	10 11	301.00	32	11 4
	11 12	344.00	36.6	12
	12 13	820.00	87.2	13
	13 14	208.00	22.1	14
	14 15	531.00	56.4	15
	15 16	321.00	34.1	16
	16 17	254.00	27.0	17

3/24/95 KC

pool fr 9, 10 = 6 ml total
↳ = 317,250 units loaded onto Blue Sepharose

unit def:
unit/ul → inc. 10,000 pmole at 30min ⇒ ~~2027~~ $\frac{216\text{ pmol}}{2\text{ul}} = \frac{108\text{ pmol}}{\text{ul}} \times 1000 \times$ = 1.08

$1.08 \times 10^5 \frac{\text{pmol}}{\text{ul}} \left(\frac{30\text{min}}{10\text{min}}\right) = 3.24 \times 10^5 \frac{\text{pmole}}{\text{ul}}$

$\frac{10{,}000\ \text{pmole}}{\text{unit}}$

= 32.4 u/ul x 300

= 97,200 units

T Page

Witness d & Und rst d by m ,	Date	Inv nted by	Dat
		R cord d by	7-20-95

g 'N

Cut sepharose

Wash for 9, 10 of S200 (P10?) V_f = 6 ml

1 col vol/hr = 10 ml/hr = 0.17 ml/min

Wash O/N

7 col vol buffer B total = 70 ml

(0.08 ml/min x 70 ml in 15 hr)

collect 10 x 7 ml fractions of wash

77 min/fr

HOD 5 BANK 2

.00	CONC %B	0.0
.00	CONC %B	0.0
.00	NL/MIN	0.08
.00	PORT.SET	6.1
.00	PORT.SET	6.1
.00	VALUE.POS	1.1
.00	VALUE.POS	2.1
.00	CONC %B	0.0
.00	ML/MIN	0.08

HIL. M=5 B=2
11.31 RE= 11.31

gradient: will scale down gradient for TPI of P182, 9 &

for 10 ml Blue col

200 ml total gradient (20 col vol)

of 50mM - 1M KCl

3 col vol/hr = 0.17 ml/min

3 ml/fr

6 min/fr

1 mm/min

C Combs
7/21/95

start ~ 7:45 AM

To Page No.

d & Understood by me,	Date	Invented by	Date
		Record by Carolyn Combs	7-20-95 7-21-95

g No.__

Cut sepharose

Load for 9, 10 of S200 (P10F) V_e = 6 ml

1 col vol/hr = 10 ml/hr = 0.17 ml/min

Wash O/N

7 col vol buffer B total = 70 ml

(0.07 ml/min = 70 ml in 15 hr)

collect 10 × 7 ml fractions of wash

7 min/fr

HOD 5 BANK 2

.00	CONC %B	0.0
.00	CONC %B	0.0
.00	NL/MIN	0.08
.00	PORT.SET	6.1
.00	PORT.SET	6.1
.00	VALUE:POS	1.1
.00	VALUE.POS	2.1
.00	CONC %B	0.0
.00	ML/MIN	0.08

FAIL. M=5 B=2
11.31 RE= 11.31

gradient: will scale down gradient for TFI of P 182, 9 ø

for 10 ml Blue col

200 ml total gradient (20 col vol)

of 50 mM - 1 M KCl

3 col vol/hr = 0.17 ml/min

3 ml/fr

6 min/fr

1 mm/min

C. Combs
7/21/95

start ~ 7:45 AM

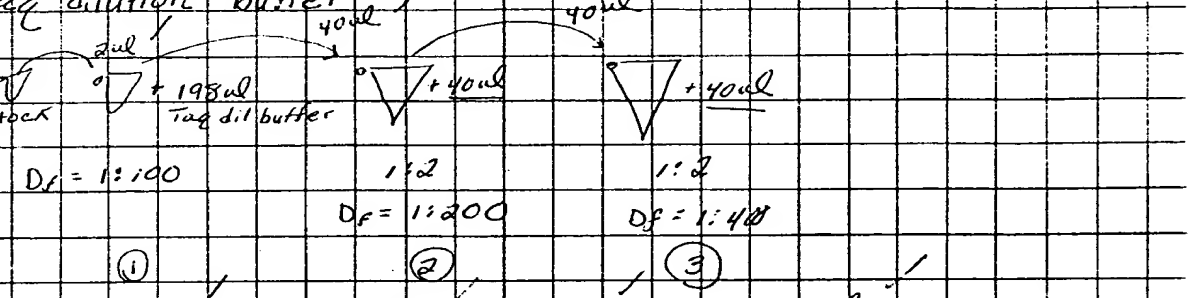
To Page No.____

& Understood by me,	Date	Invented by	Date
		Recorded by Carolyn Combs	7-20-95 7-21-95

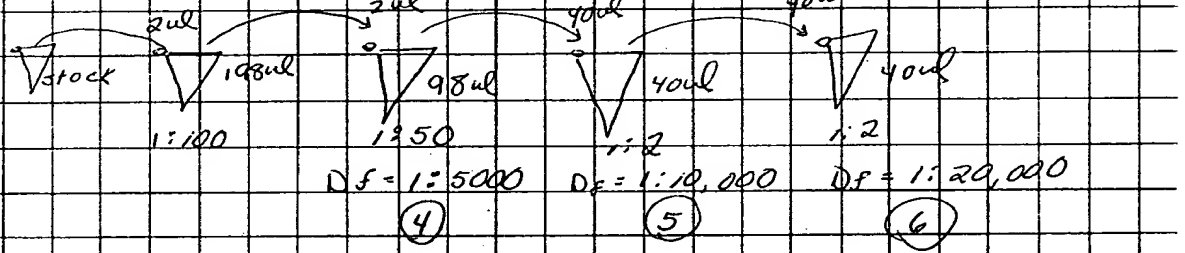
From Pag No.__ 7/21 Unit assay ^{32}P α dCTP ref 7/14/95

dilutions in Taq dilution buffer

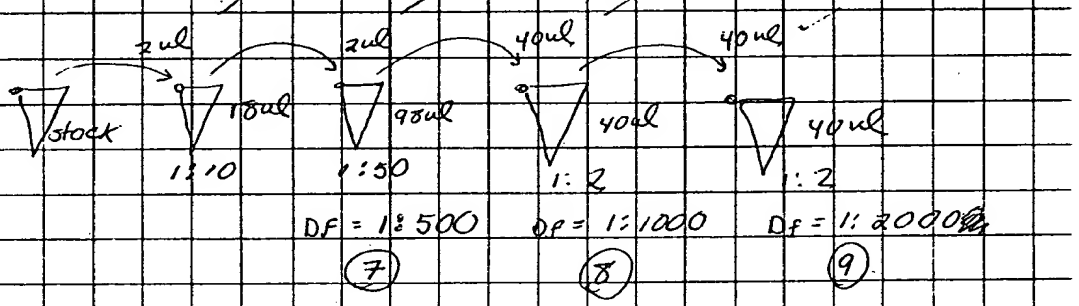
- Fr I '/PE I 355mL



- Am SO4 pellet
- 0.89mL resuspension



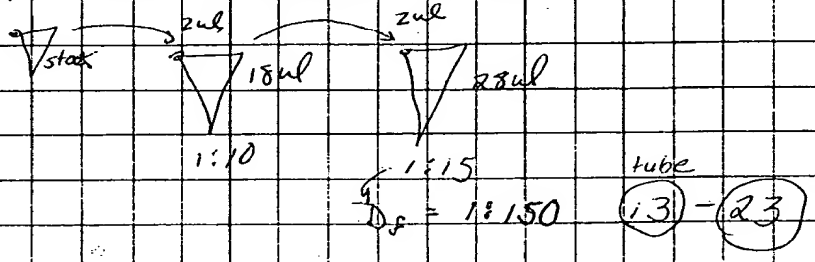
- S200 pooled peak fractions 6mL



- Fractions from Blue Sepharose columns

undiluted wash fractions W5 (10)
W10 (11)
F5 (12)

peak elution fractions 10-20



Start rxns by adding 2ul of each dilution to 48ul A mix p. 102
74°C 10min
+10ul 0.5m CDTA, spot & wash ~~spot 20ul~~

T Pag N

Witnessed & Understo d by m ,	Date	Invented by	Dat
[signature]	7/24/95	R c rd d by Pauline Ponta	7/21/95

e N .__

	CPM1	pmole / cpm / specific activity
1100	4504.00	
1200	3334.00	
1400	1539.00	
1000	11945.00	
0,000	8088.00	
0000	4497.00	
500	9437.00	
1000	5329.00	
2000	3011.00	
ash 5	108.00	
ash 16	98.00	
r 5	107.00	
10	651.00	
11	813.00	
12	1642.00	
13	3668.00	
14	7866.00	
15	10,929.00	
16	6668.00	
17	6788.00	
18	5668.00	
19	3724.00	
20	2935.00	

frx 13-17

2 ml (of 3 ml) from each fraction V_f = 10 ml

Analysis of Urea prot

Dialyze against 1000 ml buffer D (pH 8.0)

continued on p. 112

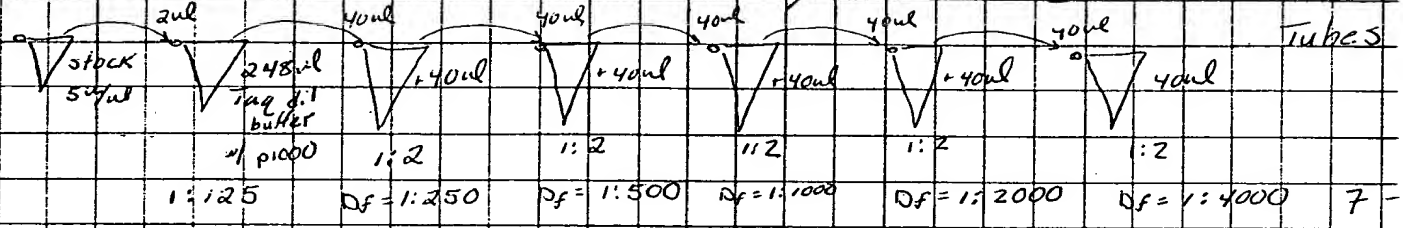
To Page No._____

I & Understood by me,	Date	Invented by	Date
Solange B	7/21/77	Recorded by Carolyn Combs	7-21-77

From Page No. Unit assay on old Tne prep (5-7-95) side-by-side the new Tne pool which was eluted from Blue ~~agarose~~ Sepharos column on 7/21/95 p.111 NB 11.

enzyme dilutions in Taq dilution buffer:

Tne (5-7-95 Liz) - ~~take~~ previously determined to be 5 u/ul, but i have lost activity over time



7-
+ n

same dilutions also made w/ Tne from Blue Sepharose column pool

start rxns w/ 2ul of each dilution into 48ul mix A ref 0.102
10' 74°C, stop w/ 10ul 0.5M EDTA, spot 20ul
^{32}P αdCTP ref 7/14/95

	SAM	CPM1	pmol	u/ul	
Tne (5-7-95 Liz)	1 1/125	2730.00	352	5.44	6.41
	2 1/250	1638.00	290 (343)		7.60
	3 1/500	790.00	202		7.00
	4 1/1000	388.00	95		
	5 1/2000	232.00			
	6 1/4000	143.00			
Tne (7/14/95 Blue Sepharose pool)	7 1/125	9450.00			
	8 1/250	5021.00			
	9 1/500	2872.00			
	10 1/1000	1669.00	207	31.0	32.5 avg
	11 1/2000	899.00	107	32	
	12 1/4000	503.00	56	33.8	
	13 no enz	67.00			

C. Cumb 7/22/95

~~23.2~~
~~22.2~~ cpm/pmol
as of 7-22-95

ave 7.0 u/ul

(= 193600 units total in blue pool fr 5-17)

T Pag

Witn ss d & Understo d by m ,	Dat	Inv nt d by	Date
	7/24/95		7-22-95
		R cord d by	

PAGE 113 OF NOTEBOOK WAS BLANK

[redacted]

Project No.________

Book No.________ TITLE Heparin column (same P…

continued from P111

From Page No.

check conductivity after dialysis of P111

10 μl / 1 ml H…

buffer D 88 μS

Hep col effluent 84 μS

Dialysate 94 μS

∴ conductivity is good and is similar to P183,9 for TF1

1. Load on ~22 ml Hep equilibrated op… with buffer D (P100)

at 0.67 ml/min (= 40 ml/hr = 2 col vol/h…

2. wash 1 col vol

Gradient (start ~9:30AM)

50 mM - 1.05 M KCl (0.67 ml/…

using buffer D and E (2M KCl)

∴ 0-50% E

600 ml total gradient vol (~30 col vol)

∴ 50-700 mM KCl is in 20 col vol same a… P185,9

0.5 mm/min span = 2A

To Page No.

Witnessed & Understood by me, [signature] Date 7/24/91

Invented by [signature] Date 7.22.91

Recorded by

Storage buffer

Page No.

2 L buffer F (as per 91342.PRP)

2	Tris pH 8	40 ml ✓
5	mM EDTA	0.4 ml ✓
1	mM DTT	2 ml ✓
0	2% NP40 Pierce	✓
0	1% Tween 20 Pierce	✓
	Glycerol	1 L ✓
	H_2O	
		2 L

Dialyze vs 1 L for 3 hr. Change to another L for O/N

ut assay on fractions eluted from Heparin column:
do 1 dilution (1/150) of fractions ~~#~~ 35-53 and a series of dilutions on the fraction with the maximum UV absorbance - #44

; dilutions in Taq dilution buffer

2ul stock → 298ul Taq dilution buffer P1000

1:150 for fractions 35-53, called H35-H53

and ~~#115~~, 1:250, 1:500, 1:1000, 1:2000, 1:4000 of H44 & 1:8000 - see dilutions on p 112.

do make 1:125 but don't do rxn w/ it

To Page No.

ed & Understood by me,

Polcari

Date 7/24/95

Invented by

Recorded by

Date 7-27-95

[redacted]

Project No.______

Book No.______ TITLE ______

From Page No.__

				pmol	u/µl
fr 44	1	1:250	2180.00	274	10.3
	2	1:500	938.00	114	8.5
	3	1:1000	560.00	55	5.8
	4	1:2000	398.00	44	
	5	1:4000	252.00		
	6	1:8000	146.00		
no Enz	7		57.00		
fr 35	8		1024.00		
36	9		699.00		
37	10		1128.00		
38	11		1127.00		
39	12		2104.00	pmole	
40	13		2484.00		
41	14		2621.00	412	9.27
42	15		4760.00	748	16.82
43	16		3657.00	574	12.92
44	17		3507.00	550	12.39
45	18		4717.00	740	16.7
46	19		4120.00	647	14.6
47	20		3517.00	552	12.4
48	21		3280.00	515	11.6
49	22		2282.00		
50	23		1820.00		
51	24		1301.00		
52	25		750.00		
53	26		513.00		

} ave 9.53 u/µl

specific activi[ty] = 19.1 cpm/[p]

Hep fr 1:150 dilution 35–53

$\frac{412 \text{ pmole}}{2 \text{ ul}} \times 150 \times \frac{30}{10} / 10{,}000 = 9.27$ u/ul

$\bar{x} = 13.3$ u/ul ± 2.6 u/ul

pool 41–48

(8 frns) (1.34 ml/frn)

= 10.72 ml total

pooled frn 41–48 (~10.7 ml total vol)

Dialyze into buffer E

see P 115

Recovered 2.6 ml The after dialysis (in by[...]

add 2.6 ml buffer G of 4-29-95 (see P 6, 10 and 9 13 42 -)

T Pag N[o]

Witn ssed & Understo d by m , [signature] Dat 7/24/97

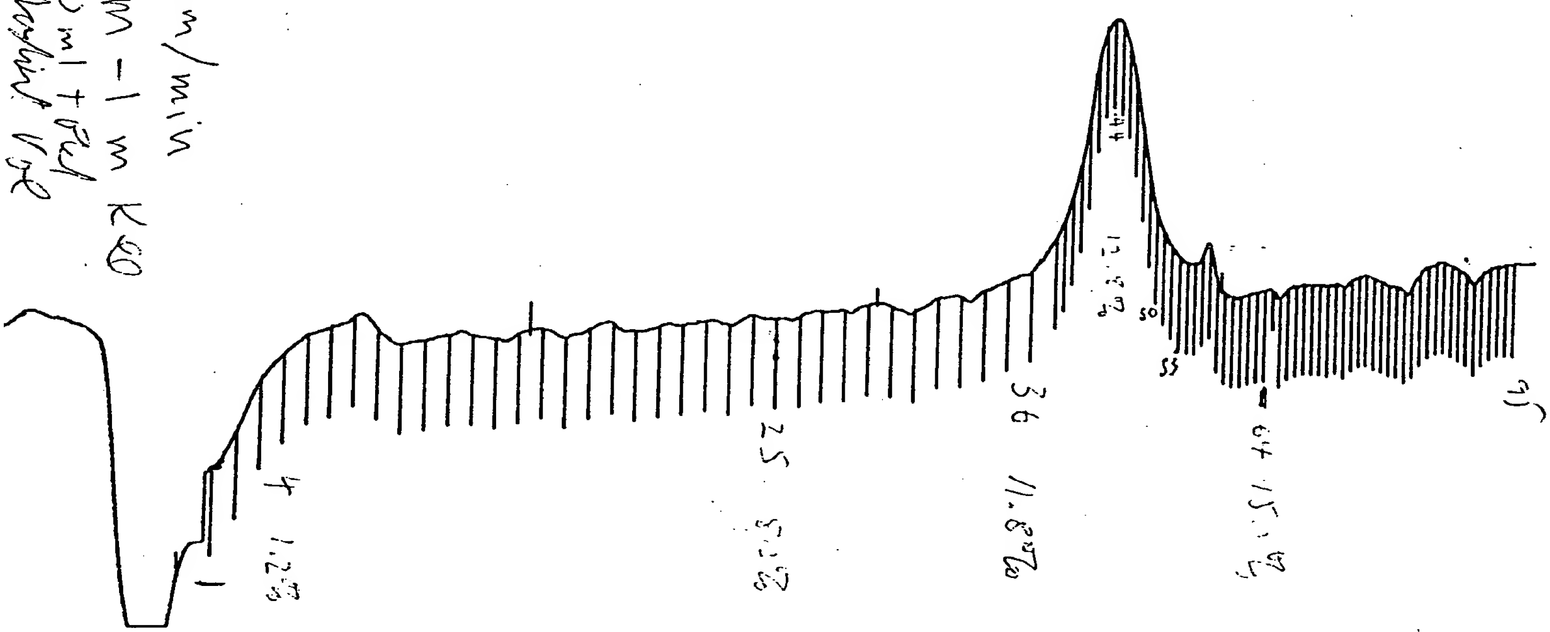
Inv nt d by [signature] Dat 7-22-95

R c rded by

7-22-95 24 0.67 ml/min
20 ml Heparin TNE
7-22-95 Load

0.5 mm/min
50 mM – 1 M KCl
600 ml total
gradient vol

2 min fr



ed & Understood by me,

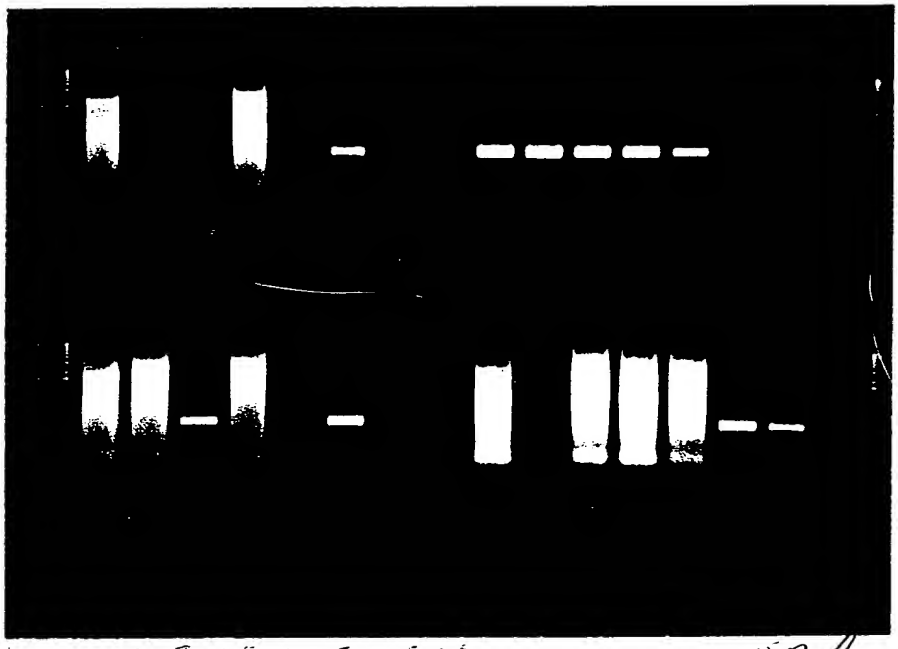
Date 7/24/95	Invented by	Date 7-22-95	
	Recorded by		

From Page No.__

This experiment is detailed on p. 119-120, NB 11

The Taq
J-7-95 Liz

50 ul rxns units 3 2.5 2 1.5 1 0.5 0.25 0.125



.75 3 2.5 2 1.5 1 .5 .25 .125

7-27-95

Blue Heparin
Seph

Witnessed & Und rst d by me,	Dat	Inv nted by	Dat
	7/24/95	R cord d by	7-27-95

PCR rxns with Blue Sepharose and Heparin fractions of Tne prep 7/22/95

g N — 7/23/95

tion of Blue Sepharose pool fractions and Heparin pool fraction of Tne. aq and Tne (5-7-95) prep will be tested in PCR alongside the new Tne. n we use more than 2 units of new Tne and not get a smear? e 5-7-95 Tne prep gives a smear w/ more than 1 unit (p. 83 NB11)

ions:
heng buffer
5 cycles w/ old program ie lab 15 9600 #76 94°C 1 min
st 0.125, 0.25, 0.5, 1, 1.5, 2, 2.5, 3 units 50ul rxn 35x { 94°C 30sec; 55°C 30sec; 72°C 2 min } 4°C
ke the 350 bp product

cktail w/ all components except enzyme, for 34 rxns

340 ul	5x Cheng
34 ul	10 mM dNTPs
34 ul	50 pg/ul M13 RF
34 ul	20 uM anchor primer > see p. 42 NB11
34 ul	20 uM 6681 primer
1156 ul	H_2O house distilled
1632 ul	→ 48 ul / PCR tube for 9600

zyme dilutions in Taq SB:

r Tne (5-7-95 liq) and rTaq - both 5 u/ul do the following dilutions

stock 5 u/ul	+23.3 ul Taq SB (10 ul)	+4 ul (20 ul)	+5 ul (20 ul)	+6.6 ul (20 ul)	+10 ul (20 ul)	+10 ul (10 ul)	+10 ul (10 ul)	+10 ul (10 ul)
Df =	1.5 u/ul	1.25 u/ul	1 u/ul	0.75 u/ul	0.5 u/ul	0.25 u/ul	0.125 u/ul	0.0625 u/ul

start rxns w/ 2 ul of each dilution on ice, flick, spin down

r Blue sepharose pool - 23.1 u/ul when normalized to 5-7-95 Tne
ie. $\frac{5}{7}(32.3) = 23.1$ p. 112, NB11

stock 23.1 u/ul → (10 ul) 36.2 ul Taq SB → 1:4.62 → 5 u/ul

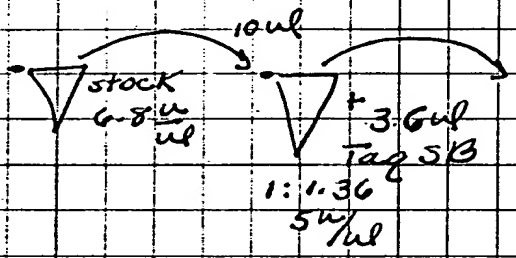
same as Tne (5-7-95) dilutions

To Page No.

ed & Und rst od by me, Polanyi	Date 8/1/95	Invented by	Date
		Recorded by Carolyn Comb	7/27/95

From Page N .__

Heparin fraction #44, which had peak UV absorbance is 6.8 u/ul when normalized to Tne (5-7-95 prep) ie $\frac{5}{7}(9.53) = 6.8$ u/ul p. 116 NB 11



same dilutions as for Tne (5-7-95) p

start rxns w/ 2ul of each dilution starting w/ the 1.5 u/ul dilutions

35 cycles, stop w/ EDTA stop soln, 7.5ul
run 25ul on gel

Result on p. 118 NB 11 - new Tne prep (7-22-95) is not less prone to making a smear than the old (5-7-95) prep. So, DNA contamination of enz should not be Tne's main problem. Rather, Tne may have an intrinsic activity that makes it "smear" more easily than Taq

T Pag I

Witness d & Und rstood by me, Dat 8/1/95 Inv nt d by Dat 7/27/95 R corded by

Fr m Page No.__

1). Can rTaq make a smear in high Mg^{2+} ? ± primers ± temp

mix A = for 8 rxns (8 units)

~~494.4~~ ~~510.4ul~~ H_2O
80 ul 10x PCR buffer ✓
→ 16 ul 10mM dNTPs ✓
16 ul 50 pg/ul M13 RF target ✓
16 ul 20uM anchor ✓
16 ul 6681 primer, 20uM ✓
~~1.31~~ ~~1.6ul~~ rTaq ~~5 u/ul~~ 8.28 u/ul
.29ul H_2O ✓
640ul

mix B = for 8 rxns

542.4ul H_2O
80 ul 10x PCR b
16 ul 10mM dNTP
~~1.31ul~~ ~~1.6ul~~ rTaq 5 u/
.29ul H_2O ✓
640ul ✓

start rxns by adding Mg^{2+}

#	1 (underlined)	2	3	4	5	6	7	8	9	10	11	12	13
H_2O	17.9	17	16	12	8	4	0	17.9	17	16	12	8	4

mix A ✓ = + primers template: 80 ——

mix B ✓ no primers no template: 80 ——

add last just before PCR

50mM $MgCl_2$	2.1	3	4	8	12	16	20	2.1	3	4	8	12	16

100ul rxns

35 cycles
stop whole rxn w/ 11 ul stop soln w/ 10x EDTA p 79 ✓

To Pag N

Witnessed & Understood by me, [signature]

Date 8/1/95

Inv nt d by [signature]

Record d by Pamela Pomb

Dat 7/27/95

N

xes Tne work better (make more product) with a hot start?

mix C for 4.5 rxns = 90 ul 5x Cheng ✓
301.5 ul H_2O ✓
9 ul 50 pg/ul m13 RF ✓ in TC 50 from 2/94
9 ul 20 uM anchor primer ✓
9 ul 20 uM 6681 primer ✓
9 ul 10mM dNTPS ✓
427.5

R Tne 7/22/95
real 19.0 u/ul
1:30.5x dilution
6 : 183 7/22/95 pic p.
6 ul new Tne
177 ul Taq SB

95 ul + 5 ul Tne 0.2 u/ul hot start (15)

duplicate hot start (16)

cold start duplicates (17) (18)

stop at 25, 30, 35 cycles
20 ul + 3 ul stop
~~11 ul stop~~

added enz after 1' 94°C denaturation → HSX 2

Can human spleen genomic DNA promote smear formation - bad seed?
no template, no primers

high Mg^{2+} - short smear condition
2.5 rxns

25 ul 10x PCR buffer ✓
187.5 ul H_2O ✓
5 ul 10mM dNTPS ✓
7.5 ul 50mM $MgCl_2$ ✓
225 ul

mix E

Low Mg^{2+} - long smear condition

25 ul 10x PCR buffer ✓
189.75 ul H_2O ✓
5 ul 10mM dNTPS ✓
5.25 ul 50mM $MgCl_2$ ✓
225 ul

2 ul D ✓
5 ul H_2O ✓
5 ul genomic DNA ✓ 80 ng/ul TC/5
5 ul Tne, 0.2 u/ul (19)

90 ul D ✓
5 ul H_2O ✓
5 ul Tne (20)

90 ul E ✓
2.5 ul H_2O ✓
2.5 ul genomic DNA ✓
5 ul Tne (21)

90 ul E ✓
5 ul H_2O ✓
5 ul Tne (22)

remove 10 ul at 15, 20, 25, 30, 35 cycles STOP tubes + 2 ul stop

To Page No.

d & Understood by me, Solange
Date 8/1/95
Invented by
Recorded by Evelyn Combs
Date 7/27/95

[redacted]

124 Project No.______
Book No.______ TITLE __________________

From Page No.__

4) Does Tne make a smear when 1 or 2 dNTPs are missin[g] from rxn?

4 dNTP mix, 2.5mM each ⇒ 20ul each 10mM dNTP stock (T ✓ C ✓ G ✓ A ✓)

3 dNTP mix, AGC 2.5mM each ⇒ 20ul 10mM A ✓
20ul 10mM G ✓
20ul 10mM C ✓
20ul H_2O ✓

2 dNTP mix, GT 2.5mM each ⇒ 20ul 10mM G ✓
20ul 10mM T ✓
40ul H_2O ✓

(use 8ul of mixes i[n] each 100 PCR rxn for C_F = 2[...])

mix F (for 20 rxns) = 200ul 10x PCR buffer ✓
1480ul H_2O ✓
1680ul

(for 9.5 rxns)

G: 798ul ✓
28.5ul 50mM $MgCl_2$ ✓
883.5ul

H: 798ul ✓
19.95ul 50mM M[gCl_2] ✓
8.55ul H_2O ✓
883.5ul

I	J	K	L	M	N
261ul G 24ul 4dNTP	261ul G 24ul 3dNTP = 3dNTP mix	261ul 24ul 2dNTP			
23, 24	25, 26	27, 28	29, 30	31, 32	33, 3[4]

95ul
+5ul Tne .24/l
100ul

35 cycles
11ul stop (p. 79)

Lab 16, 9600 method 103 1:25pm

T Pag N

Witnessed & Understood by me, [signature] Dat 8/1/95
Invented by [signature]
Recorded by [signature] Dat 7/27/95

ge No.__

2% agarose gel ⇒ 3.6g agarose
300mL 1X TAE
20ul EtBr 5989

rTaq

	target + primers						no input DNA					
$MgCl_2$	1.05	1.5	2	4	6	8	10	1.05	1.5	2	4	6	8	10

l. Comb 7/25/95

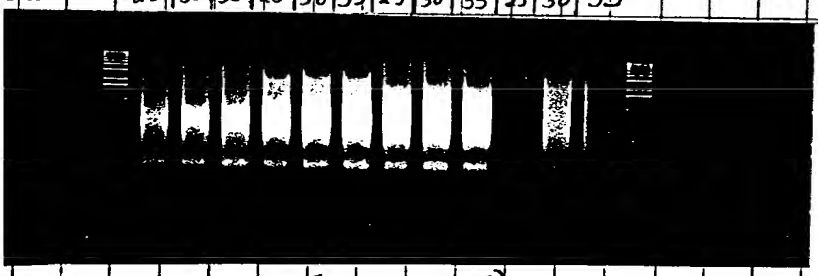
top
1 - 14 ⇒ 25ul
15 - 18 12 tubes 20 cycles inc. cycles 2
20 tubes 19 - 22 ⇒ 10ul inc cycles ie 19 15C 19 20C 19 25C ect
bottom
23 - 34 ⇒ 25ul

Results

- Increasing Mg^{2+} did not cause rTaq to make a smear either in the presence or absence of input DNA

Tne (7-22-95 prep) ⟶ Cheng buffer primers + target 3ul/10ul rxn

	hot start			cold start			hot start			cold start		
cles	25	30	35	25	30	35	25	30	35	25	30	35

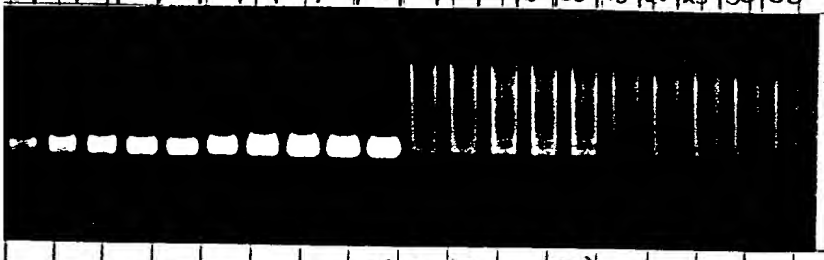


l. Comb 7/28/95

- With 3units Tne, the hot start rxns did not smear any less than cold start rxn. I should have used 1.36 units in order to get product instead of smear
redo this expt w/ 1.36units Tne rxn

Tne (7-22-95)

	1.5mM $MgCl_2$							1.05 mM $MgCl_2$							PCR buffer 3ul/rxn ⇒ too much
	+ genomic					− genomic		+ genomic					− genomic		
	15	20	25	30	35	15	20	25	30	35	15	20	25	30	35	15	20	25	30	35



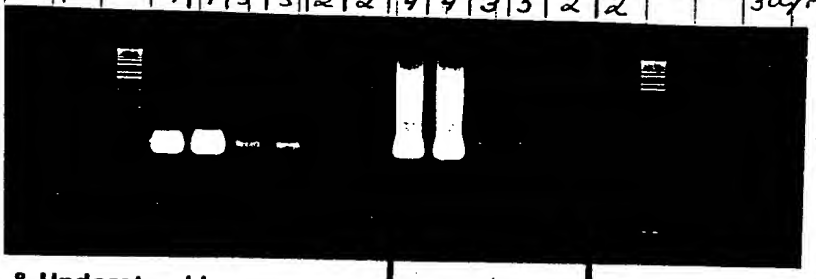
l. Comb 7/27/95

- Addition of genomic DNA did not result in less smear over time w/ 3units Tne

redo this expt w/ 1.36units Tne rxn

Tne (7-22-95)

	1.5mM $MgCl_2$						1.05 mM $MgCl_2$						→ PCR buffer 35 cycles 3ul/rxn
esent	4	4	3	3	2	2	4	4	3	3	2	2	



l. Comb 7/25/95

3 dNTP mix = AGC present
2 dNTP mix = GT present

- No smear made when 2 dNTPs (GC or A) are missing, so smear is probably not made by a TdT activity.

To Page No.____

& Understood by me,
Polanyo

Date 8/1/95

Invented by

Recorded by Carolyn Comb

Date 7/27/95

Project No._____

126 Book No._____ TITLE _____

From Page No.__

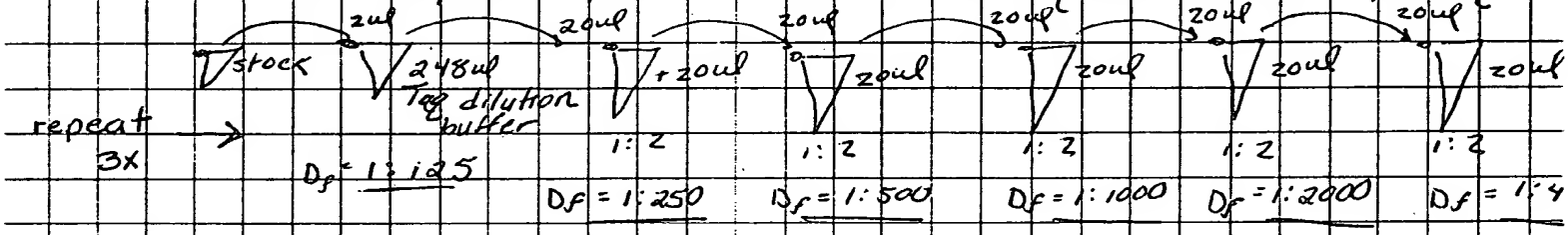
7/26/95 Unit assay of new Tne prep (7-22-95) after dialysis into Taq storage buffer.

- old 5-7-95 Tne prep & rTaq will be done too
- 3 replicates of each dilution series, done 3x independ

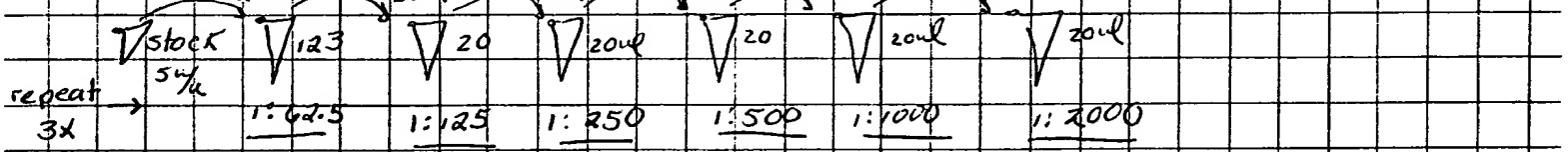
cocktail: 206 ul of Taps, $MgCl_2$, KCl mix –
2,520 ul H_2O w/ p1000
60 ul 10mm dNTPs
405 ul 3.7 mg/mL gapped DNA
9.3 ul ^{32}P αdCTP ref date 7/14/95
3.2mL

use 48 ul per rxn
start rxns by adding 2ul of enzyme dilutions on ice.

Dilutions of ^Heparin pooled fraction ~~rTaq 5B~~ final dilute w/ Taq di



Dilutions of Tne (Liz 5-7-95) and rTaq both labeled 5 u/ul



- rxns stopped w/ 10ul 0.5M EDTA
- 20ul of each rxn spotted on GFC filters →

T Pag N

Witn ssed & Understood by m ,	Dat	Invented by	Dat
[signature]	8/1/95	[signature]	7/27/95
		R cord d by Dan Pomb	

E. Combs 7/27/95

	SAM	CPM1	ave pmol	ave u/µl	
	1	8081.00			
	2	7346.00	625	11.7	
	3	5871.00			
	4	5139.00			
	5	5341.00	441	16.5	
	6	4559.00			
	7	3009.00			
	8	2963.00	255	19.1	
	9	2724.00			
	10	1492.00			
1/1000	11	1359.00	125	18.8	
	12	1429.00			
	13	891.00			
1/2000	14	899.00	79		
	15	895.00			
	16	490.00			
1/4000	17	402.00			
	18	524.00			
	19	6160.00			
1/62.5	20	6476.00	582	5.4	
	21	7195.00			
	22	4215.00			
1/125	23	4266.00	354	6.6	
	24	3596.00			
	7	2124.00			
1/250	8	2014.00	181	6.8	
	9	2055.00			
	10	1160.00			
1/500	11	998.00	95	7.0	
	12	1024.00			
	13	572.00			
1/1000	14	610.00			
	15	609.00			
	16	361.00			
1/2000	17	352.00			
	18	348.00			
	19	8453.00			
1/62.5	20	6925.00	658	6.17	
	21	7075.00			
	22	4769.00			
1/125	23	3803.00	387	7.25	
	24	4613.00			
	25	2896.00			
1/250	26	2565.00	240	9.00	
	27	2722.00			
	28	1185.00			
1/500	29	1404.00	115	8.60	
	30	1334.00			
	31	1234.00			
1/1000	32	873.00	89.7	13.40	
	33	953.00			
	34	592.00			
1/2000	35	527.00			
	36	509.00			
	37	56483.00			
	38	57656.00	56855 ave	= 34.1 cpm/pmol	
	39	56427.00			

Tne 7-22-95

19.0 u/λ ave

⇒ use (x ul)(19.0) = 1.36 u/100 ul

⇒ 0.0716 ul/100ul PCR rxn

⇒ we had been using 0.2 ul/100ul rxn
0.2 ul is really 1.36 units, not 1 unit as we thought based on the unit value 5 u/ul

Tne Liz

6.8 u/λ ave

expected ~5 u/λ

Tne

ave 8.28

expected 5 u/λ or ~9 pu/λ real units in nmol/50'

Tne

⇒ use (x ul)(8.28 u/ul) = 1.36 u/100ul PCR

= 0.164 ul/100ul Taq

To Page No._____

Understood by me,	Date	Invented by	Date
Bolanp	8/11/95	Recorded by Carolyn Combs	7/27/95

dU containing templates

"Fidel pri" (27 mer) 7-11-95

Exhibit 141
Appl. No. 09/558,421

GAGACATGGCGTCCCAGTCACGACCT
CTCTGTACCGCAGGGTCAGTGCTGGACTAGTACGAGCTACT
U

27 bp

"Fidel Temp
or "Fidel Temp
(7-11-95)
(42-mer

#51351 → This is not old "Fidel T
of pJ1, 10. This new ou
less hairpin or primer
according to oligo prop

. ssDNA region is same as MG JBC Creighton & MG
for + dGTP + dATP (get G-A mismatch at position 3 and)
+ dCTP for rescue

. For test of dATP incorp opposite Template dU,
have all 4 dNTPs present at 200 μM each
and look for pause one site before dU
(run on 20% PAGE?)

```
      G
      C
      A ↑
  U   T A
      G C  (31)
      A T
      T A
      C G
primer  (27)
```


Witnessed & Understood by me,	Date	Invented by	Dat
Deeraen Bolup	8/1/95	Recorded by	7-28-95

Project No.________ dU vs dT in template

56 Book No.________ TITLE

From Page No.__

	1	2	3	4	5	6	7	8	
Ppurified Temp dT / 50nM primer (P57)	4 →								10 nM / ~1pm total
Ppurified Temp dU / 250nM primer (P55)					4 →				
10X Taq PCR buffer	10	10			10	10			1.5m
50mM MgCl	3	3			3	3			
10X Vent buffer			10	10			10	10	
10mM dNTPs	2 μl →								200 dNTP
rTaq 0.0625 u/λ	2				2				
Tne 0.0625 u/λ		2				2			0.125 / 2 to
Vent 0.0625 u/λ			2				2		0.013 pm pol mi
DeepVent 0.0625				2				2	
H_2O	79 →		82 →		79 →		82 →		primer / pol so large primer overpol
	100 μl								

preheat to 70°C
Start with addition of pol.

remove 10 μl to 5 μl cycle seq stop at

0 5 10 20 40 60 90

T Page No

Witnessed & Understood by me, [signature] Date 8/1/95 Invented by [signature] Date 7-28-95

Recorded by

ge N

sequence Rxn same as P 27, 4 and 90 11
using 32P primed Temp with dT and dA

To Page No.

ed & Understood by me,

Dat 8/11/95

Invented by

Recorded by

Date 7-28-95

Project No.

128 Book No. TITLE Effect of annealing temperature on Tne smear

From Pag No.

- anneal at 50°C, 60°C, 70°C w/ 72°C extension - no input DNA
- take out rxn aliquots after 5, 10, 15, 20, 25, 30, 35 cycles
  → use 1.36 units Tne (5-7-95 Liz) / 100ul rxn
- test the effect of annealing temp on both the long (low Mg^{2+}) and short (hi Mg^{2+}) smears

mix A = high Mg^{2+} for small smear - enough for 3.5 rxns

280 ul	H_2O
35 ul	10x PCR buffer ✓
7 ul	10mM dNTPs C_f = 200uM ✓
10.5 ul	50mM $MgCl_2$ C_f = 1.5mM ✓
332.5 ul	

mix B = low Mg^{2+} for long smear - enough for 3.5 rxn

283.15 ul	H_2O
35 ul	10x PCR buffer ✓
7 ul	10mM dNTPs
7.35 ul	50mM $MgCl_2$ ✓ C_f = 1.05 mM
332.5 ul	

* 25 fold dilutio
Tne stock (Liz
5 u/ul (real units
p.127

3ul Tne
72ul Taq
75 ul of 0.

annealing temp: 50°C Lab 15 | 60°C Lab 16 | ~~70°C~~ 72°C Lab 562

rxn #	1	2	3	4	5	6
✓ ul Mix A (hi mg)	95		95		95	
✓ ul Mix B (low mg)		95		95		95
* ul Tne 0.2u/ul (5-7-95 Liz)	5	→	→	→	→	→
	100ul rxns					

6 ← 5% received mix that were made up a different time t the mix A+B for 1

remove 10ul of rxn to 2ul STOP soln w/ EDTA p. 79 NB 11 at cycles 10, 15, 20, 25, 30, 35

To Pag

Witness d & Understo d by me,	Dat	Inv nted by	Dat
[signature]	8/1/95	R cord d by [signature]	7/28/95

g N .___

:ling   Lab 15 program 76   94° 1 min
35x { 94° 30sec
50° 30sec
72° 2 min }
4°C —

Lab 16 program 103   94° 1 min
4.5 min per cycle
35x { 94° 30sec
60° 30sec
72° 2 min }
4° —

SGI program 133   94° 1 min
~~72° 2 min 30sec~~
2 temp. PCR   35x { 94° 30 sec
72° 2 min 30 sec }
4° —

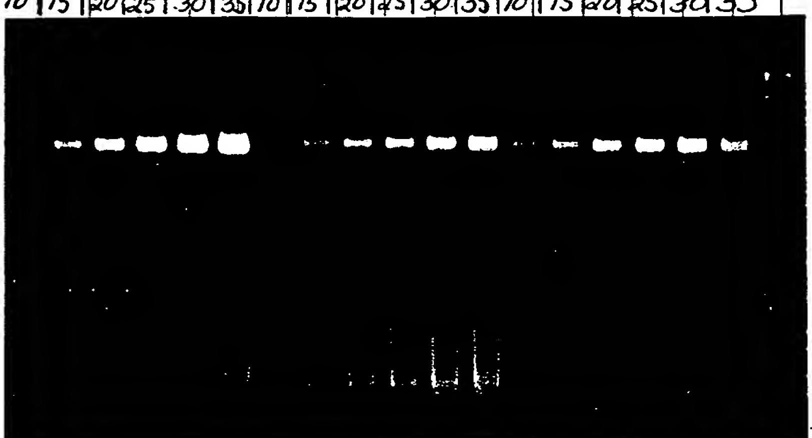
2% agarose gel, 36 samples + primers [2ul 20uM anchor, 2ul 20uM 4481, 94ul H_2O, 11ul stop] + uncycled Tne [1.5ul Tne Lig, 36ul 513, 5ul, 95ul H_2O, 11ul stop] (load 20ul

short: 50° 1-10 to 1-35; 60° 3-10 to 3-35; 72° 5-10 - 5-35 } load 20ul
long: 50° 2-10 to 2-35; 60° 4-10 to 4-35; 72° 6-10 to 6-35 }
primers, 7/5/95 Tne, 7/22/95 Tne

Tne (5-7-95 Lig) 1.36u/rxn

ng   50°C   60°C   72°C
10 | 15 | 20 | 25 | 30 | 35 | 10 | 15 | 20 | 25 | 30 | 35 | 10 | 15 | 20 | 25 | 30 | 35

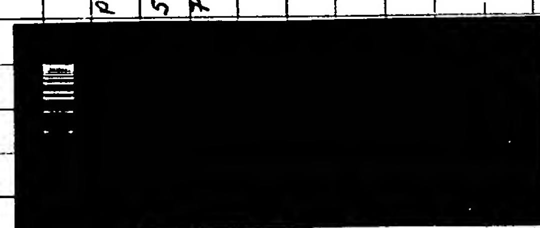
primers alone, 2nmoles anchor, 2nmoles 4481
5-7-95 Tne
7-22-95 Tne uncycled



1.5mM $MgCl_2$

1.05 mM $MgCl_2$

7/28/95



Comb 7/28/95

To Page No.______

sed & Understood by me, [signature]   Date 8/1/95
Invented by [signature]
Recorded by [signature]   Date 7/28/95

Project No._____
Book No._____

TITLE same as P121,9 and P52,10 — unit assay for 1.1X Taq

From Pag No.__

		Rxn#	ul/assay	1% Tween20/NP40 ea
4°C #10 (P121,9) (no det)		1-3	2	1 µl
11	1.1X	4-6	3.64	
1	(2X 0.1% T/N)	7-9	2	
14	(2X Tfl buffer)	10-12	2	
no Taq (same as P121,9) ½ dil		13-17	2	
~~Fidel Fort~~ 1.1X called "new" on P54 (5-8-95)		18-20	2	
Room temp #11 (1-27-95)	1.1X	21-23	3.64	
-20, 5 months on P52 (see Rxn#21-23 on P52) got 5-4% recovery (on P53)		24-26	2	

Exhibit **143**
Appl. No. 09/558,421

°C		SAM	CPM1	ave	µl/µl	% of zero time P122,9
		1	1779.00			
10		2	1970.00	2029	.008	38%
		3	2337.00			
11		4	8375.00		.035	105
		5	8284.00			
		6	8267.00			
1		7	10246.00	9784	.039	105
		8	9851.00			
		9	9556.00			
		10	8959.00			
14		11	9908.00	9484	.037	107
		12	9584.00			
		13	10530.00			
Taq		14	9527.00			
		15	9706.00	10,119	(.04 by definition)	
		16	9859.00			
		17	10773.00			
		18	6924.00			
37		19	7046.00	7065	.028	(was .025 on P53 so >100%
55		20	7219.00			
		21	6156.00			
		22	6520.00	6257	.025	77% (P154 9 is 0 time po (P38, 10)
1	5	23	6095.00			
		24	5038.00			
0°C		25	4980.00		.019	.017/.030 = 63% recovered
4-55		26	4755.00			see P53
RGD		27	82.00			
ul 5		28	119284.00			
		29	121726.00			

To Page N

Witness d & Underst d by m ,	Dat	Invented by	Date
DDPolesky	8/11/95	Record d by	7-31-95

From Page No. Is more specific product made and less smear in a hot vs cold star
Look at products at 10, 15, 20, 25, 30, 35 cycles

• start 100ul rxns w/ 2ul enz in order to keep [glycerol] low ⇒ SB
50%, rxns will be 1%, do duplicate hot & cold start rxns

• materials: ~~5x stock of Tne~~

[A] mix: (100mM Tricine pH 9, 5.25mM MgOAc, 425mM KOAc) ⇒ 5X

100ul 1M Tricine pH 9 ✓
5.25ul 1M MgOAc ✓
212.5ul 2M KOAc ✓
682.25ul H_2O ✓
1ml

cocktail for 4.5 reactions = 90ul 5X [A] mix ✓
315ul H_2O ✓
[B]
9ul 50pg/ul M13RF ✓
9ul 20μM ancha primer ✓
9ul 20μM 4681 primer ✓
9ul 10mM dNTPs ✓
441ul

98ul B + 2ul Tne 0.68u/ul
98ul B + 2ul Tne
98ul B + 2ul Tne
98ul B + 2ul Tne

① ② ③ ④

cold start

hot start - enz a dilutio denati of 1st
2:40 PM

Tne dilution: 3ul Tne (5-7-95 Liz) 4.84u/ul ✓
27ul Taq SB ✓
30ul of 0.484u/ul Tne

T Pag N

Witness d & Understood by me, Dat 8/1/95
Inv nt d by
R cord d by Pamela Pomb
Dat 7/31/95

g N.__

nove 10ul after 10, 15, 20, 25, 30, 35 cycles + add 2ul STOP w/ 100mM EDTA
rk to 9600 program 103 = 94°C 1min
35x { 94°C 30sec
55°C 30sec
72°C 2min
4°C —

% agarose gel top
— → 2% 1-10C to 1-35C thr 4-35C } 2
2% 19-10C 22-35C } 2

lt: Tne (5-7-95 Liz prep) 1.36 u/rxn

Cold Start - duplicates Hot Start - duplicates

cycles	10	15	20	25	30	35	10	15	20	25	30	35	10	15	20	25	30	35	10	15	20	25	30	35

buffer: 20mM Tricine
85mM KOAc
1.05mM MgOA

3.1 uL

- 380bp m.3 product

lusion: Hot start did not result in more specific product.
One cold start duplicate run failed, no apparent reason.

To Page No._____

ed & Understood by me,	Date	Invented by	Date
Bolanos	8/1/95	Recorded by Carolyn Cumby	7/31/95

Project No._____

32 Book No._____ TITLE Addition of genomic DNA to a Tne PCR rxn

rom Page No. 123

same as p. 123 #3 except only 1.36u of Tne (5-7-95 will be used per rxn

#19 90ul D ✓
5.5ul H_2O ✓
2.5ul genomic DNA 5/4/95 HS#2 ✓
2ul Tne 0.68u/ul p.130

#20 90ul D ✓
8ul H_2O ✓
2ul Tne

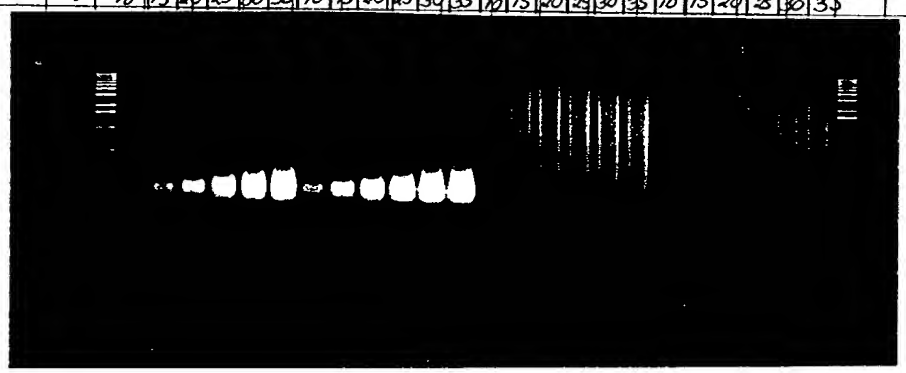
#21 90ul E
5.5ul H_2
2.5ul g
2ul Tne

#22 90ul E ✓
8ul H_2O ✓
2ul Tne

10ul aliquots removed after 10, 15, 20, 25, 30, 35 cycles + 2ul stop w/ 100m

Tne 1.36 u/rxn (5-7-95 Liz prep) - no pr

esult: prese



7/31/95 CC

Conclusion: 1) The smear is visible even after 10 cycles - we shou
run aliquots of earlier cycles to determine when the
smear becomes visible
2) The 1.05 mM Mg^{2+} long smears are more intense with
genomic DNA present than without genomic DNA. T
ay be a real result or it may be variation in rxns - repeat w/ triplicate

of support for
a that genomic
DA can exist as
a bad seed.

To Pag N

n ssed & Understood by me,	Date	Invent d by	Dat
	8/1/95	Record d by Paulson Pomb	7/31/95

Mg^{2+}] titration in a short PCR rxn w/ Tne (5/7/95 Liz)

Page No. N

Purpose: To demonstrate that lowering [Mg^{2+}] shifts the size of DNA product in smear from small to longer ~~size~~ in a short PCR rxn w/ Tne alone. This result has been observed w/ ~~Tc~~ 1.5mM Mg^{2+} vs 1.05mM Mg^{2+}, but the range of concentrations has not been tested. Does the transition occur over a narrow or broad range of Mg^{2+}? What happens w/ 85mM KOAc & 1.5mM Mg^{2+} & we have not tested this condition before

Final expt conditions:

[ ] MgOAc (mM) 0.9, 1.05, 1.1, 1.15, 1.2, 1.3, 1.4, 1.5, 1.7 (9 levels)

[ ] KOAc (mM) 50, 85 (2 levels)

Template and primers for m13 330bp product present

35 cycles, 55°C annealing temp

1.36 u/rxn Tne - 5-7-95 Liz prep

Start rxns w/ Mg^{2+}

Materials: 25mM MgOAc → $\frac{25mM}{100mM} \cdot \frac{100ul}{} = \frac{25ul\ 1M\ MgOAc}{975ul\ H_2O}$

A - 50mM KOAc for 9.5 rxns

19ul 1M Tricine pH 9.0 ✓
23.75ul 2M KOAc ✓
734.35ul H_2O
19ul 10mM dNTPs (f: 200uM) ✓
19ul 20uM 6681 primer ✓
19ul 20uM anchor primer ✓
19ul 50 pg/ul M13RF ✓
1.9ul Tne (5-7-95 Liz prep) → 1.36 u/rxn (← 0.8 u/ul)
855ul

mix B - 85mM KOAc 9.5 rxns ✓

19ul 1M Tricine pH 9 ✓
40.375ul 2M KOAc ✓
717.725ul H_2O ✓
19ul 10mM dNTP ✓
19ul 20uM 6681 ✓
19ul 20uM anchor ✓
19ul 50 pg/ul M13 ✓
1.9ul Tne 5-7-95 ✓
855

	top of gel								no tube 9 - I dropped it	10 - 18	there is a tube 18	12 PM - ~3 PM Lab 16 9600 10.3
	1	2	3	4	5	6	7	8	9			
	90ul									90ul		
	6.4	5.8	5.6	5.4	5.2	4.8	4.4	4	3.2	same series as 1-9		
MgOAc	3.4	4.2	4.4	4.6	4.8	5.2	5.6	6	0.8			

add Mg, mix well & keep on ice til cycling
vortex

100ul rxns
stop w/ 1ul STOP w/ 100mM EDTA, run 20ul on 1.2% agarose gel

To Page No.____

Read & Understood by me, [signature] Date 7/7/95

Invented by [signature] Date

Recorded by [signature] 8/1/95

Addition of genomic DNA to a Tne short PCR rxn - 3, 6, 10, 12, 15, 20 cycle aliquot

g N .132

rpose: To determine if adding human genomic DNA to a Tne PCR rxn leads to production of a smear by an earlier cycle # than without genomic DNA. Genomic DNA (200 ng/100 ul rxn) might act like more "bad seed" material and exacerbate the smearing rxn. This experiment was tried on p. 132. Cycle #'s 10-35 were run on the gel. There was an indication that addition of genomic DNA made the smear darker by an earlier cycle #. Now, we are repeating the p. 132 exp in triplicate and looking at even earlier cycle #s.

mmary of experimental cond: 3 rxns w/ human spleen DNA, 3 without
terials: no primers added
start rxns by adding 1.3 ul/100 ul rxn w/ Tne (5-7-95 Lig prep)
@ 55°C annealing temp program 103, Lab 16
200 ng of human spleen DNA/rxn

terials: mix A: for 7.5 reactions = 75 ul 10x PCR buffer ✓
569.25 ul H_2O ✓
15 ul 10 mM dNTPs ✓
15.75 ul 50 mM $MgCl_2$ ✓

Tne (5-7-95 dilution
2 ul Tne
18 ul SB
20 ul 0.6 Tne

top of gel — bottom of gel

	1	2	3	4	5	6
90 ul	—	—	✓	—	—	✓
5.5 ul	—	—	✓	8 ul —	—	✓
c.DNA 2.5 ul	—	—	✓	none —	—	✓
7 ul						
(0.68 ng/ul) 2 ul	—	—	—	—	—	—
-7-95 Lig prep						

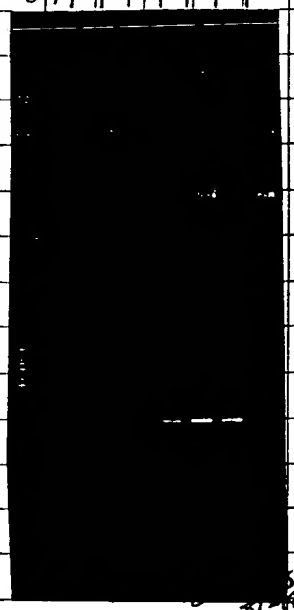
ve 10 ul to a tube on ice w/ 2 ul STOP soln in it (100 mM EDTA)
3, 6, 10, 12, 15, 20 cycles, run 10 ul on 1.2% agarose gel
= 6-9: + 3 ul 6-9C went into 6-9C stop tube C = cycle #
See result on p. 134

To Page No. 134

ed & Understood by me,	Date	Invented by	Date
Sarah A Roberts	5/7/95		
		Recorded by Carolyn Coons	8/1/95

TNE (5-7-95 Liz prep) 1.36 u/rxn, 100ul rxn w/ template + primers

MgOAc (mM) 0.9 | 1.0 | 1.1 | 1.15 | 1.2 | 1.3 | 1.4 | 1.5



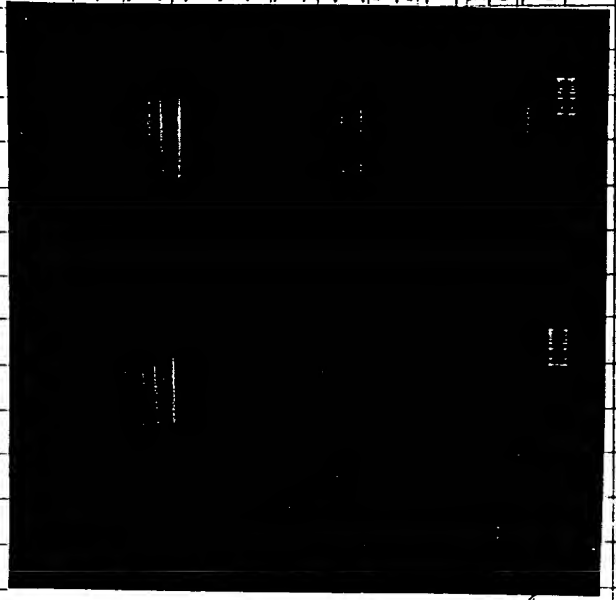
50mM KOAc

85mM KOAc.

- 1.3mM MgOAc was optimal for making product - note that we have made prc w/ 1.05mM Mg^{2+} in earlier e when glycerol > 5% p.86
- 50mM KOAc is not sufficient for produ formation, but 85mM KOAc is - valu between 50 & 85 have not been testec
- The size of DNA products in sme does vary from small to longer as $[Mg^{2+}]$ varies from 1.5 - 0.9mM Mg

triplicate rxns w/ TNE (5-7-95 Liz prep) (see p135 for reaction)

	replicate 1						replicate 2						replicate 3					
cycle #	3	6	10	12	15	20	3	6	10	12	15	20	3	6	10	12	15	20



+ 200ng human spleen genomic DNA per 100ul rxn

no genomic DNA

Conclusion:
There maybe a l amount of contam DNA still in RNA in TNE prep - 3 cycles needed see smear.

e.e combs 8/2/95

T Pag

Witn ss d & Underst od by m , Dat 8/7/95 Inv nt d by Dat 8-2-95

R c rded by Sandra Combs

Project No._____

136 Book No._____ TITLE Mg^{2+} titration in Long PCR w/ Taq: Tne

From Page No.__

purpose: To determine if Mg^{2+} controls long PCR smear size and to see if the smear is primer/template independent. Note that Long PCR Kit uses different buffering components than we've been using for Si

In a single Tne enz PCR of 330 bp product, 1.2mM Mg^{2+} = 1.3mM is at the center of transition from small to long smear is optimal for product formation if 85mM KOAc present.

do: 7 levels of Mg^{2+}, ± target and primers, 2 ratios of Taq: Tne

materials: LTI:S Taq Long PCR system + Kalas recommendations for 7-22-95 Tne prep

dilution of Tne in Taq SB: 2ul Tne (7-22-95 prep, 19 u/ul)

① 59.64ul Taq SB ✓ 1:30.8 ✓

mix

② 2ul of dilution ①

59.64ul Taq SB 1:30.8 ✓

61.64ul of 0.024/ul Tne

enzyme mixes:

final Taq (u):	Tne (mu)	Taq (5u/ul)	Tne (0.024/u)	SB
→ 1	1	4 10	4 2.5	5 3
1	10	4 10	40 25	8 1
1	2	2 5	4 2.5	7 1

mix 1 with primer and template for 16 rxns: 16ul 10mM dNTP ✓

16ul primer mix 1 ✓

KSU 2 DNA 1 2mg/ml, 8ul BSA H2O, 40ul genomic temp

272ul H_2O ✓

320ul

mix 1 without primer + template, 16 rxns:

16ul 10mM dNTP ✓

304ul H_2O ✓

320ul

T Pag N

Witn ss d & Und rst d by m ,	Dat	Inv nt d by	Dat
Doreen Bokup	8/7/95		
		R c rd d by Sandra Pauls	8/2/95

g N. —

Mg²⁺ mM

2 (1:1 TT) 1 for 5 rxns = 50 ul 5x A ✓

Taq:Tne 0 30 ul 5x B 2.5 ul

5 5 ul 1u Taq : 1mU Tne (7-22-95 R.L. prep)

95 95 ul H_2O

150

2 (1:1 TT) 1.1 mM Mg^{2+} = 45 ul 5x A ✓

5 ul 5x B ✓

5 ul 1u Taq : 1mU Tne ⇒ (~~a 100ul H_2O enz mix~~

add H_2O 1st 95 ul H_2O → add)

2 (1:1 TT) 1.2 mM Mg^{2+} = 40 ul 5x A ✓

10 ul 5x B ✓ → ~~Barry's aliquots~~

5 ul 1u Taq : 1mU Tne

95 ul H_2O

2 (1:1 TT) 1.3 mM Mg^{2+} = 35 ul 5x A ✓

15 ul 5x B ✓

5 ul 1u Taq : 1mU Tne

95 ul H_2O

2 (1:1 TT) 1.4 mM Mg^{2+} 30 ul 5x A ✓

20 ul 5x B ✓

5 ul 1u Taq : 1mU Tne

95 ul H_2O

2 (1:1 TT 1.5 mM Mg^{2+} 25 ul 5x A

25 ul 5x B

"

2 (1:1 TT) 1.6 mM Mg^{2+} 20 ul 5x A

30 ul 5x B

"

1 mix 2

ke each of these mixes again with the 1:10 TT mix

d a mix 2 (1:2 TT) 1.6 mM Mg^{2+}

To Page No.____

ed & Understood by me, [signature] Date 8/7/95

Invented by [signature]

Recorded by [signature] Date 8/2/95

138

Project No.

Book No. **TITLE**

From Page No.

#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
mix 1 w/ template w/ primers	20														20														20
mix 1 no template primers								20														20							
mix 2 (1:1) 1	30							30																					
1.1		30							30																				
1.2			30							30																			
1.3				30							30																		
1.4					30							30																	
1.5						30							30																
1.6							30							30															
mix 2 (1:10) 1															30							30							
1.1																30							30						
1.2																	30							30					
1.3																		30							30				
1.4																			30							30			
1.5																				30							30		
1.6																					30							30	
mix 2 (1:2) 1.6																													30

10:30am Lab 15 94°C 30sec
35 cycles { 94°C 30sec, 60°C 30sec ~6.5hr, 68°C 10min }
4°C

stop w/ 7 µl stop soln containing 100mM EDTA (p.79)
7 µl run on 0.7% agarose gel, 190V 6:30-7pm

T Pag N

Witnessed & Understood by me,	Date	Invented by	Date
Deborah Polang	8/7/95	[signature]	
		Recorded by Diolm Ponds	8/2/95

Project No._____ 32 P end label Pri 2 --- Pri 2
140 Book No._____ TITLE for Test of decreasing primer lengths

From Page No.___

mix for 14 labeling rxns: 23 ul $^{3-2}$P ATP (3.33 uM) ref 8/4/95 (6.66 pmol per
25 ul 5x kinase buffer
4.2 ul Pol K - new lot
50.56 ul H_2O
146 ul

rxns = 1.66 ul of each oligo - Fidel pri 2, 3, 4, 6, 8, 10, 12, 14, 16, 1
← 10 uM stocks
(16.6 pmol primer tot
8.34 ul mix
10 ul in 9600 tubes
37°, 30' in Lab 14 9600
55°C, 5' "
cool to 4°C before opening tubes

add 2 ul 10 uM Fidel temp dT = 20 pmol total
80°C, 5'
cool to Room temp, 15 min in 9600

add 64.7 ul 10 mM Tris pH 8.0
stored at -20°C overnight

use 4 λ/100 λ reaction for 10 mM primer

template / pri = 20 pmol / 16.6 pmole

To Pag N

Witn ss d & Und rsto d by m , Dat 8/7/95 Inv nt d by Dat 8-7-95
R cord d by

Exhibit 147
Appl. No. 09/558,421

142

Project No.
Book No.

TITLE Test extension of short primers
Δ [Taq], Δ length of primer

From Page No.

	1	2	3 (not done)	4	5	6	7	8	9	10	11	12	13	14	15	16	17
^{32}P fid pri 6 - fide/Temp	2 →					→											
" 8							2 →										
" 10													2 →				
" 12																	
" 16																	
" 20																	

(P140, 250 nM primer)

Mix A 46 μl ——

Taq μ/l ~~sil buffer~~ 5B

Taq μ/l	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
.0016	2						2						2				
.008		2						2						2			
.04			2						2						2		
.2				2						2						2	
1					2						2						2
5						2						2					

50 μl

70°C ~~20~~ min 22 min
stops with 25 μl cycle seq stop solution
load 3 μl on 25% PAGE
(same as P155, 7)
2000 V (gets ~11 mA) for 3 hr

.0032 units (eg. tube #1) 21 pmol nt in 20 min
(based on units & max efficiency)

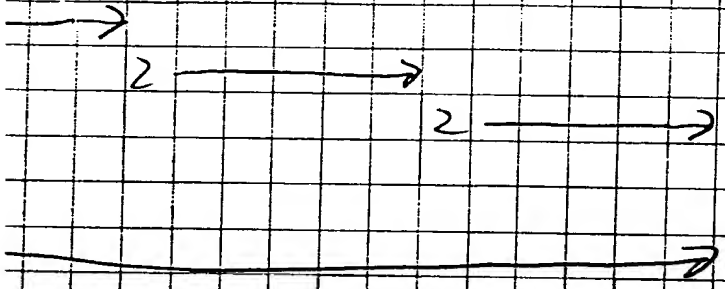
input primer is 1 pmol 42 mer
= ~20 pmol nt incorp for 20 nt ssDNA seq

T Pag N

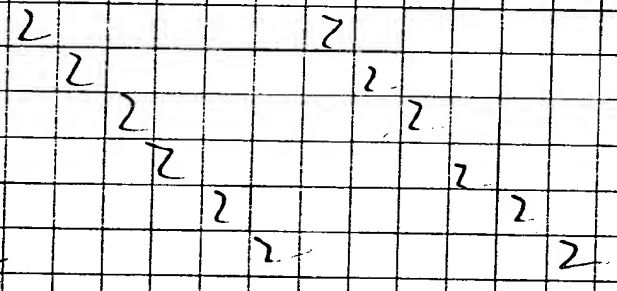
Witnessed & Understood by me, [signature]
Date 8/7/95
Invented by [signature]
Recorded by [signature]
Date 8-3-95

g No.__

22 23 24 25 26 27 28 29 30 31 32 33 34 35 36



10 nM primer Cf
(1 pmol primer total/50 µl Rx



(for 40 Rxns)

Mix A

H2O
10X PCR buffer
50 mM $MgCl_2$
10 mM dNTPs

1.5-40 ml
200
60
40
1.940 ml

use 46 µl/Rxn

Cf = 1.5 mM $MgCl_2$

200 µM dNTP Cf in Rxn.

To Page No._____

& Understood by me,
Natalia Polanyi

Date
8/7/95

Invent d by

Recorded by
Carolyn Combs

Date
8-7-95

PAGE 144 OF NOTEBOOK WAS BLANK

g N.

Results:

1. primer length 10 and longer are extended by Tag.

2. Will Test Tne vs Tag next to see if Tne does better than Tag

To Page No.

ed & Understood by me,

Date 8/14/95

Invented by

Recorded by

Date 8-3-95

rom Page No.__

32P fid pri 6 · fidel Temp
" 8
" 10
" 12
" 14
" 16

(P140 250 nM primer) *preheated to 70°C w/mix A*

Tne diluted in SB 7/22/95 R.L.
2.0 1.56 u/l
0.0625 u/l
0.25 u/l
1 u/l

Taq 5/31/95 Lin
0.0156 u/l
0.0625 u/l
0.25 u/l
1 u/l

Mix A (P.143) 46 µl
Vf = 50 µl

20 min 70°C stop with 25 µl cycle seq stop solution

heat to 90°C, 5 min before loading

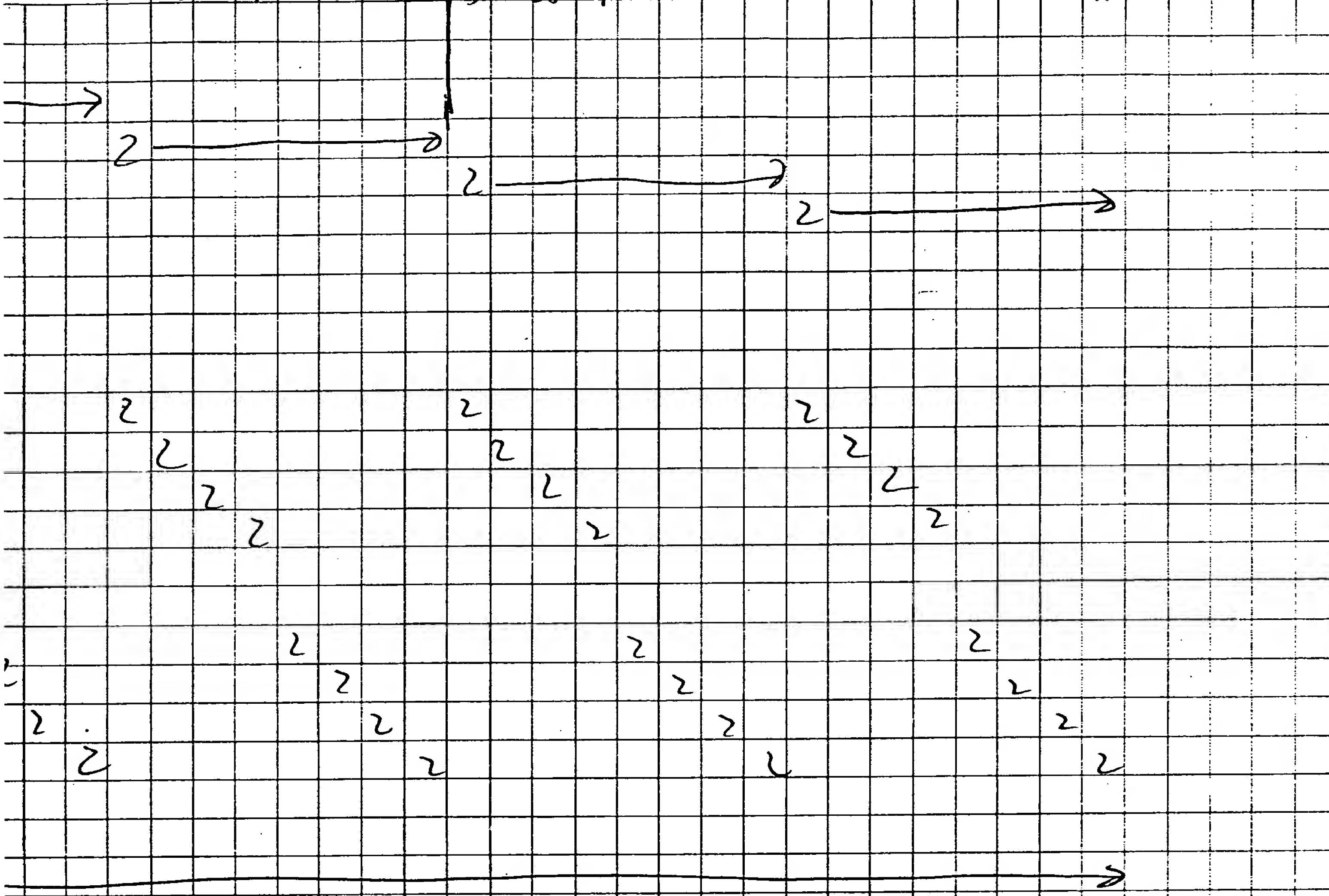
25% PAGE run as P142 and 155 ✓ ; ran 3 hr 2000 V

mix A p.143 scaled up 1.5X = 2.31 mL H_2O
300 ul 10x PCR buffer (from Kala ✓)
90 ul 50mM $MgCl_2$ ✓ (made from 1M 2/15 STO
60 ul 10mM dNTPs ✓ → 50 ul 1M + 950 ul H_2O
2760 ul

To Page No

Witness d & Understood by m,	Dat	Invented by	Dat
Dawn Rolens	8/7/95	Recorded by Dawn Rolens	8-4-95

Page No. 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48



...yme dilutions: in Taq storage buffer — 12/7/94

...aq 5u/ul stock — ~~5ul~~ 10ul → + ~~200ul~~ 40 SB — 10ul → +30ul SB — 10ul → +30ul SB — 10ul → +30ul SB

...31/95

1:5 — 1:4 — 1:4 — 1:4

1 u/ul — 0.25 u/ul — 0.0625 u/ul — 0.0156 u/ul

...ie 1st normalize to Taq units p. 127 19/8.28 x 5 = 11.47 u/ul (Taq was thought to be 5 u/ul

11.47 u/ul stock — 5ul → +52.35ul SB — 10ul → 30ul SB — 10ul → 30ul SB — 10ul → 30ul SB

1 u/ul — 0.25 u/ul — .0625 u/ul — .0156 u/ul

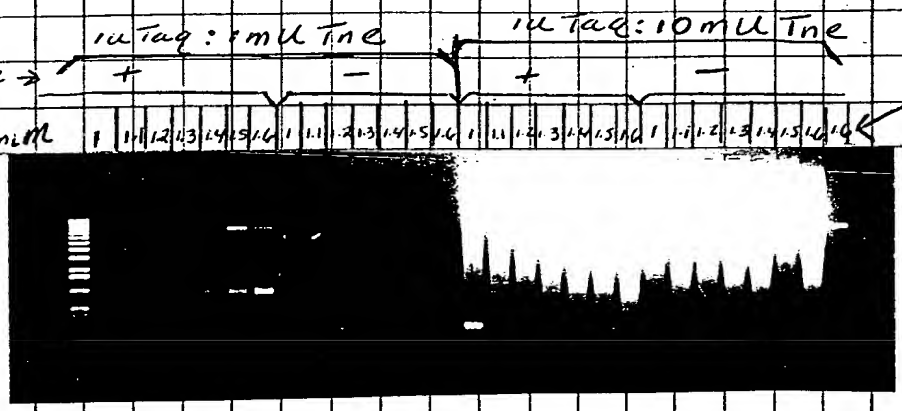
To Page No. ______

...ed & Understood by me, [signature] Date 8/7/95

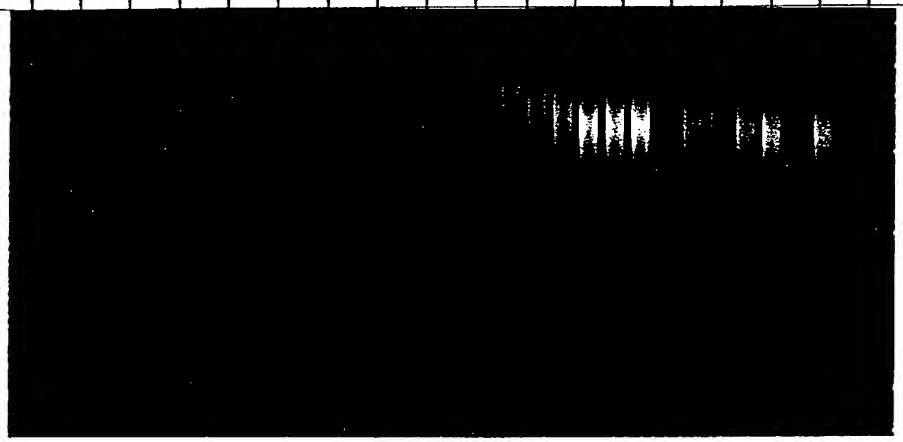
Invented by [signature] Date 8-4-95

Recorded by Carolyn [signature]

g N .__



8/15/95 cc



8/15/95 cc

There are 2 kinds of nonspecific products

1) primer/target dependent nonspecific products, which are seen with both 1munit and 10munits Tne. The products are discrete bands, mostly ≤ 1Kb. The ~~taten~~ yield of these nonspecific products, as well as the specific 7.5Kb product increase as $[Mg^{2+}]$ increases. The highest ratio of specific to nonspecific product occurs at 1.5 mM Mg

2) primed/target independent products. The products form an intense smear from wells down to ≤ 200bp. As $[Mg^{2+}]$ increases, the size of products in smear decreases and the smear becomes more intense. This is the same Mg^{2+} affect that was observed in a short PCR with Tne alone.

To Page No.____

ed & Understood by me,	Date	Invented by	Date
Rebecca Palump	8/7/95	Recorded by Carolyn Combs	8/15/95

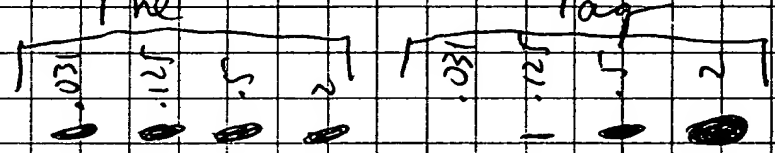
From Page No.

Results Data on P151, 152

for primer length 12 14 16 the first appearance of run off happens at 4 ÷ 16 × less Tne units than Taq units (based units based on Taq unit among P. 127

At the highest [pH], Tne and Taq have a equal extension for 12 14 nt primers and Taq had greater yield for 16 nt primer at highest level

ie. for 16 mer primer, Tne again shows first appearance of run off, but Taq has greater yield of 12 units / enzyme



∴ less Tne is needed to extend a primer. Maybe Tne is more processive That is consistent with Taq giving go extensive (equal or better than Tne) at high and could explain how Equal units of Taq, Tne for DNAse I treated gapped DNA & act differently for primer extens

To Pag

Witn ssed & Und rstood by me, [signature] Dat 5/17/95

Invented by [signature] Dat 5-7-95

R c rd d by

g N — see p. 140

d-label Fid 16: ~~4 ul~~ ^{32}P-γ-ATP ref 8/4/95
~~4~~

8.08 ul H_2O ✓
4 ul 5x Kinase buffer ✓
3.32 ul 10uM Fid 16 oligo ✓ (~~33.2~~ 16.6 pmol)
4 ul ^{32}P-γ-ATP ref 8/4/95 (3.33uM, 4.66 pmol/um)
0.6 ul PNK

20ul in 9600 tube
37°C, 30min ✓
55°C, 5min ✓
cool to ~4°C ✓

+ 4ul Fidel Temp, 10uM 42mer (40pmol)
50°C, 5min
cool for 15min to RT in PCR machine
+ 189.4 ul 10mM Tris pH 8.0

store at -20°C

To Page No.

ed & Understood by me,	Date	Invent d by	Date
Polanp	8/14/95	Recorded by Carolyn Comb	8/7/95

PAGES 150-151 OF NOTEBOOK WERE BLANK

32P 42 mer for 3'exo assay of 3'exo(-) Klenow fragment

g N._

Fidel Template 10 μM (42 mer)	5.8 μl	✓	5 Pmol total
32P ATP	1	✓	
5x Kinase buffer	1	✓	
PNK 10 u/l	2	✓	
H_2O	13.2 μl / 35	✓	

37°C, 30'

70°C, 5'

1M KPO_4 pH 7.6

1M Monobasic	1.3 ml
1M K dibasic	8.7 ml
	Vf = 10 ml

To Page No.____

d & Understood by me, Andrea Roland	Date 8/14/95	Inv nt d by [signature]	Date 8-8-95
		R corded by	

Project No.
52 Book No. TITLE Primer extension: time course with 4 [enz], Taq vs Tne Fid16 primer

rom Pag No.

purpose: In the last primer extension experiment, ~4x less Tne th Taq was able to extend the Fid16 primer to a full leng 42mer product. p.150. The highest level of Taq (2u), that was tested, made even more full-length product than the highest level Tne tested (2u). ~~Today's expt.~~ The purpose of today's e is to confirm the results of p. 150 and to determine whether Tne = Taq have different affinities for DNA/primer bi or if they are the same but Tne is better at extendin after a stop. → the time course - no enz rxn will reveal if Tne cures a stop sequencing rxns and the Fid16 primer without enzyme will also be run on the 25% gel.

materials:

PCR/Mg^{2+} mix to mix with enzyme prior to beginning rxns:

		conc
25 ul	10x PCR mix ✓	1.25x
7.5 ul	50mM $MgCl_2$ ✓	1.25x
167.5 ul	H_2O ✓	
200 ul		

(2) ✓

↳ use 16 ul ✓
+ 4 ul of enz. dilution in SB ✓
20 ul - 1x PCR buffer = 20mM Tris 8.4, 50m
tubes 1-8 1x $MgCl_2$ Cf = 1.5mM

(1) mix A for 10 rxns: 80 ul 10x PCR buffer ✓
636 ul H_2O ✓
24 ul 50mM $MgCl_2$ ✓
20 ul 10mM dNTPs ✓
40 ul ^{32}P Fid16 annealed to Fidel Temp p. 14
800 ul
Keep at 70°C

✓ enzyme dilutions in Taq storage buffer: same as on p. 147
same preps of Taq & Tne

(3)

✓ 5 ul STOP soln from cycle sequencing kit in 9600 tubes 1-40

To Page N

Witnessed & Understood by me,	Dat	Invented by	Date
DPolanp	8/14/95	Record d by Paula A. Pink	8/8/95

Project No.________
Book No.________

g N ._

#	1	2	3	4	5	6	7	8
tubes	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40
2/95	1' 2' 5' ...	all prewarmed to 70°C						
Tne mix / 5 PCR er + mg^{2+}	20							
Taq mix		20						
ul Tne mix			20					
ul Taq mix				20				
5 u/ul Tne mix					20			
5 u/ul Taq mix						20		
6 u/ul Tne mix							20	
u/ul Taq mix								20

rxns w/ 80ul of mix A prewarmed to 70°C, triterate w/ p200
ove 10ul of rxn to a 9600 tube containing 5ul STOP at
1min, 2min, 5min, 10min, 20min - on ice
t to 80°C, 5min before loading ~~3~~ 1.5 ul on 25% gel
er of loading: on 25% urea gel
H ATGC no enz ~~70~~ 31-40 ↑ 21-30 ↑ 11-20 ↑ 1-10 (not loaded) γATP

Fid16 (no enz Fid16) (no enz Fid16) (no enz Fid16)

↓ 16ul 1.25X PCR buffer
4ul storage buffer
+ 80ul mix A
↳ removed 10ul +
5ul stop - from cycle sequencing kit

diluted 10^4X to 1×10^{-3} uCi/ul
↳ .3ul
+ 2ul H_2O
+ 1ul STOP
load 1.5ul

d on 8% gel in same order loaded 1.5ul

To Page No.____

d & Understood by me, Polamp | Date 8/15/95 | Invented by | Date 8/8/95
Recorded by Carolyn Comb

Exhibit **152**
Appl. No. 09/558,421

52

Project No.______

Book No.______ TITLE ______________________

From Page No.__

Taq PCR conditions

	10 Rxns	A	B
BSA 20mg/ml	✓	2.5 µl	BSA 0.T...
1M KH_2PO_4 pH7.6	✓	25	
10X PCR buffer	✓		50
50 mM $MgCl_2$	✓	70	70
50% sterile glycerol	✓	140	
H_2O (sterile)	✓	232.5	350
^{32}P 42mer (fidel Template P61)	✓	10	10
		480	480

*see p 74-75
*Beckton-Dickinson conditions — Taq PCR conditions

^{32}P 48-mer no enz

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
(A)	48µl →																	
(B)										48µl →								
Klenow exo- lot CK041 75u/λ																		
dil 25 u/λ	2									2								
5 u/λ		2									2							
1 u/λ			2									2						
Klenow exo(-) lot EJP41 130u/λ																		
dil 25 u/λ				2									2					
5					2									2				
1						2									2			
Klenow (+) exo 0.6 u/λ							2									2		
0.06								2									2	
0.006									2									2

dilutions with T... dil buff

start 1:2...

3 hr, ...

25 µl cycle stop sol

load 3 µl 8% PA

To Page N

Witnessed & Understood by me, [signature] | Date 8/14/95 | Invented by [signature] | Date 8-9-95 | Recorded by

P63, 10

r m: Hartman, Chris
o: Lasken, Roger
c: Rashtchian, Ayoub
ubject: Exo minus Klenow
at : Monday, August 07, 1995 3:40PM
ri rity: High

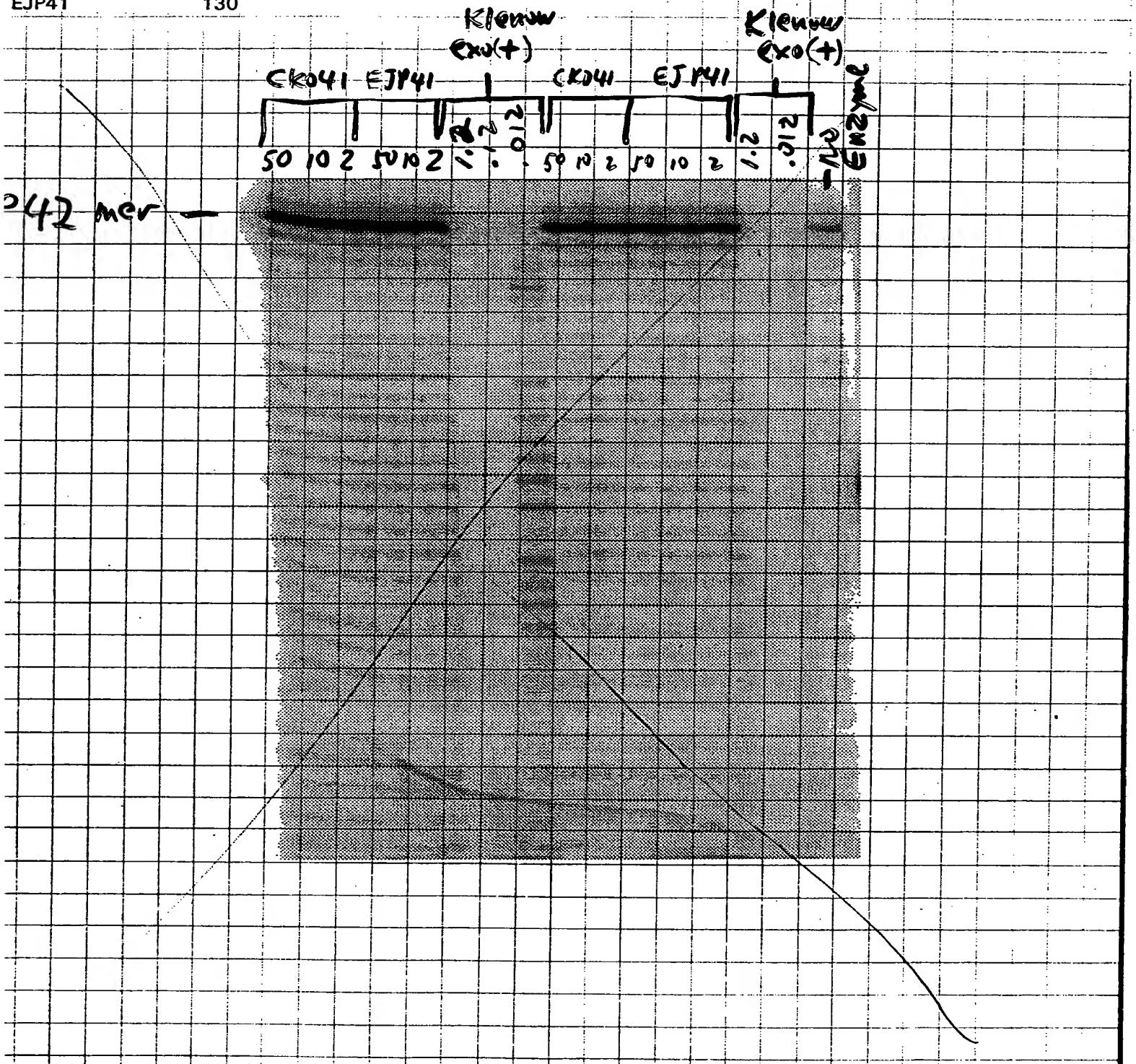
roject No._____
Book N ._____ 63

Beckton Dickinson Conditions

Taq Pol buffer

oger, The unit values for the bulk exo minus klenow are as follows:

Lot No.	U/ul
CK041	75
EJP41	130



To Page No.____

d & Und rstood by me,	Date	Invented by	Date
Polanp	8/14/95	Recorded by	8-14-95

Project No.______
Book No.______ TITLE (cold 33 correct · mp19
follow p 17, 9 for 500 pmol

From Pag No.__

33 correct (P138, 9) 20 μM	✓	~~2.18~~ 4.36 μl	~~43.6~~ 87 p[illegible]
M13 mp19 s DNA (+)	✓	200 μl	21.8 pmo[l] total
1 m Tris pH 7.5	✓	10.6 μl	(100 pri[mer]/temp[late])
	Vf	212.78 μl	50 mM Tris

∴ 0.1025 pmol circle/μl ⇒ 743 pmol nt/μl
use 2 μl/50 μl Rxn for 1.5 nmol nt/Rx[n]

Mix A (33.4 Rxns)

Taps MgCl₂ KCl (of P'120, 9)	~~103~~ μl (use 15 μl next time)	✓	
³²P dCTP 10 mCi/ml 3000 Ci/mmol	3	✓	
dATP 10 mM	33.4	✓	(200 μM) ↓
dCTP 10 mM	33.4	✓	
dGTP 10 mM	33.4	✓	
rTaq 5 u/μl	66.8 μl	✓	10 u/50
33· mp19 743 pmol nt/μl	66.8	✓	1.5 nmol nt
H_2O	1.163 ml	✓	
	1.503 ml		

use 45 μl/50 μl Rxn

T Pag

Witnessed & Understood by me, [signature] Dat 8/14/95
Invented by [signature] Dat 8-15-95
R corded by

ge N			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	2[illegible]

A 4 µl ——————————————→

CC703
β —
M 5, 5, 5, 5, 5, 5

3707 H_2O
L µl —— —
M 40 → 40 5
40 → 40 5
40 → 40 5
40 → 40 5
25 → 50 5

82 80.3 µl + 5 µl 10 mM 4 dNTP 5

(stability study began at 4°C on 1-20-95 ∴ samples are 6 months and 20 days old)

(+Taq) P 121.9
40 → 40 5
40 → 40 5
40 → 40 5
40 → 40 5
25 → 50 5

~~1070~~ µl + 30 µl ~~200~~ µM in mix B 220 (@ 1.1X) 5

A (-) Taq — 5 5 5 5 5

spot 20 µl on GFC

0 5 5 5 5

Vf = 50 µl assemble on ice → 1 min 70°C → [illegible] → 10 µl 0.5M EDTA

To Page No.____

ssed & Understood by me, [signature] Date 8/14/95
Invented by [signature] Date 8-1-95
Recorded by

Project No. _____ TITLE Results P6T

Synth. Book No. _____

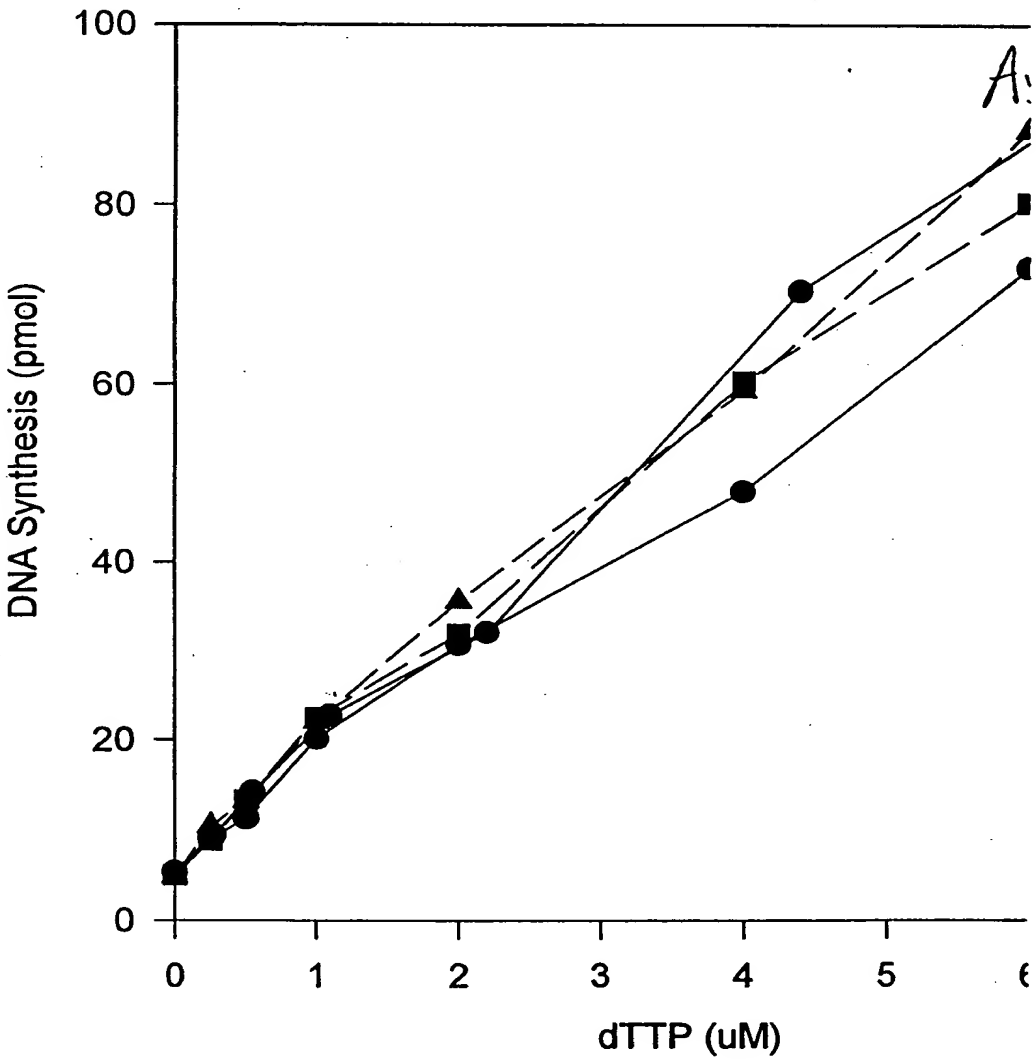
		(pmol)		
0	82.60	5.4	0	
.25	137.80	9.1	12.5	18%
.5	172.00	11.4	25	11%
1	303.80	20.1	50	10
2	463.40	30.7	100	7.5
4	725.00	48	200	6
6	1103.80	73	300	6

assuming 1/4 incorp is T (ie 9.1 pmol / 4)

1.5 pmol at max

∴ 35 = 5% on 352 at max

0	76.00	5.0
.25	134.40	8.9
.5	201.00	13.3
1	339.20	22.4
2	482.00	31.9
4	910.20	60.3
6	1211.80	80.2
0	82.20	5.4
-	145.80	9.6
.5	216.20	14.3
1	344.60	22.8
2	485.00	32.1
4	1062.00	70.3
6	1408.00	93.2
0	74.00	4.9
.25	158.40	10.5
.5	201.00	13.3
1	332.60	22
2	538.20	35.6
4	896.00	59.3
6	1333.20	88.3
40	57.20	
X	72558.92	



Wil rstood by me, Dat 8/14/95 Invented by Dat 8-9-95

R corded by

To Pag

Processivity of Taq, Tne, and Ultma

[...]g N.__

[e]xtension of 33-mer correct primer annealed to m13mp19 ssDNA
serial enz dilutions, 2 min extension and 1 10min endpt extension
[...]units – 0.0078 units in 50ul/rxns, reactions started w/ 2ul enz.
[...]/95

[Re]action cocktail for 35 rxns = 175 ul 10x PCR buffer
1347.5 ul H_2O
52.5 ul 50mM $MgCl_2$
35 ul 10mM dNTPs
70 ul ^{32}P-33mer correct annealed to m13mp19 – the Kinase rxn was done as on p. 12 NB 10, then 66 ul of m13 ssDNA added 0.26 ug/ul m13 stock
1680 ul ("^{32}P33 correct + mp19")

[No]te: 42 ^{32}P primer : 1 m13 circle
* mistake, see 8/11/95
were 2ul of the labeled & annealed primer was diluted w/ 70ul m13

This was 42 pri / circle – see P/64
where more mp19 added to get 1 pri/circle

[En]zyme dilutions in Taq SB

Taq, 5/31/95, 5 u/ul — 2 fold dilutions

5 u/ul → 4ul + 16 ul SB (1:5) → 10ul + 10ul SB (1:2) → 10ul + 10ul SB → ...

1 u/ul | 0.5 u/ul | 0.25 u/ul | 0.125 | 0.0625 | 0.0313 | 0.0156 | 0.0078, 0.0039 u/ul

[u]nit assay on 7/31

[T]ne 7/31/95, 11.47 u/ul – this value is normalized (to Taq p. 147) RL's Tne of 7-22-95

11.47 u/ul → 5ul + 52.35 ul SB → then, serial dilutions made in the same way as for Taq

1:11.47
= 1 u/ul

Ultma 6 u/ul — Lot 0643 12/31/95, Perkin Elmer

6 u/ul → 4ul + 20ul SB → 1 u/ul → same dilutions as for Taq + Tne, but Ultma units are not normalized to Taq units.

To Page No.____

[Witness]ed & Understood by me, [signature] Date 8/14/95
Invented by
Recorded by Carolyn [signature] Date 8/9/95

Project No._____

156 Book No._____ TITLE _____

From Page No.__

- ~~a~~ 48ul mix in a 9600 PCR tube, preheated to 70°C
- reactions were started by adding 2ul of enz w/ P2 and triturating w/ P200
- after 2 min at 70°C, in 9600, rxns were stopped w/ 25ul Cycle sequencing Stop solution and kept at -20°C overnight prior to loading on 8% gel

Tubes	1 — 10	11 — 20	21 — 30
	Taq	Tne	Tma
	0.0078 → 20units	0.0078 → 20units	0.0078 - 20uni

The 20units rxns were incubated for ~~20~~ 10 min; while all the other rxns were incubated for 2 min

T Pag N

Witness d & Underst d by m ,	Dat	Inv nt d by	Dat
	8/14/95	R cord d by Dawn Pomb	8/9/95

Project No._____

158 Book No._____ TITLE Extension of 16-mer by Taq + Tne with Δ MgCl + Δ KCl

From Page No.__

?

general overview of conditions tested:

Fix $MgCl_2$	Δ KCl (mM)			
1.05 mM	0	25	50	85
1.5 mM	0	25	50	85

Fix KCl 50mM	Δ $MgCl_2$ (mM)
	1, 1.2, 1.5

each condition tested w/ 0.0312u Taq + Tne
2.0u Taq + Tne
in 50ul rxns at 72° for 20min

= 44 rxns

for 1, 50ul rxn: 1ul 1M Tris ~~8.4~~ 8.5 Cf = 20mM * note that real PCR buffer is pH 8.4
41.08ul H_2O
1.42ul 3M KCl for 85mM Cf
1.5ul 50mM $MgCl_2$ for 1.5mM Cf
1ul 10mM dNTP Cf = 200uM
*2ul ^{32}P 16mer on Fid Temp Cf = 10nM
2ul enz to start rxn
50ul

* End-label 16mer as on p. 149

mix A for 50 rxns: 50ul 1M Tris, pH 8.5 ✓ ✓ 22.5 ✓
1875ul H_2O ✓ ✓ 843.75
50ul 10mM dNTP ✓ ✓ 2 10ls FHC 705 Fe 370 22.5 ✓
100ul ^{32}P 16mer annealed to Fid Temp ✓
2075ul

T Pag N

Witn ss d & Understood by m ,	Date	Inv nt d by	Dat
DDPolano	8/14/95	Record d by Daralon Pomb	8/10/95

No.__

the expt w/ varied KCl

B w/ 1.05mm $MgCl_2$
rxns
705.5ul A
17.85ul 50mM $MgCl_2$ ✓✓
7.65ul H_2O ✓✓
731ul

rxn → 180.6ul B + 21ul H_2O ✓ → C_f=0mM KCl → 48ul/rxn ✓
180.6ul B + 21ul 250.6mM KCl → C_f=25mM KCl /rxn ✓ (tubes)
180.6ul B + 21ul 501.2mM KCl → C_f=50mM KCl
180.6ul B ✓ + 21ul 852mM KCl → C_f=85mM

C w/ 1.05mM $MgCl_2$
705.5ul A
25.5ul 50mM $MgCl_2$ ✓
731ul

same but use 180.6ul C for each

Serial dilution of 3M KCl stock: ✓

3M —(50ul)→ +126ul H_2O → 852mM —(100ul)→ +70ul H_2O → 501.2mM —(100ul)→ +100ul H_2O → 250.6mM

3M

To Page No.____

& Understood by me,
Date 8/14/95
Invented by
Recorded by
Dat 5.16.95

From Page No.

for expt w/ varied $[Mg^{2+}]$'s

for 14 rxns [D] = 581 ul A
11.62 ul 3m KCl ✓ C_f = 50mM KCl/rxn
9.33 ul H_2O ✓
602 ul

for 4.2 rxns

180.6 ul D
+ 21 ul 10mM Mg^{2+} ✓
↑ use 48 ul/rxn
C_f = 1mM Mg^{2+}/rxn

180.6 ul D
+ 21 ul 12mM Mg ✓
C_f = 1.2mM Mg/rxn

180.6 ul D
+ 21 ul 15mM Mg^{2+}
C_f = 1.5mM Mg

dil of 50mM MgCl stock: ✓

50mM → 50 ul + 116.5 ul H_2O → 15mM

100 ul + 25 ul H_2O → 12mM

100 ul + 20 ul H_2O → 10mM

Enzyme dilutions in Taq storage buffer: (SB)

Taq, 5/3/95 stock 5 u/ul (hot real units)

5 u/ul → 5 ul + 40 ul SB → 1 u/ul → 10 ul + 63 ul SB → 0.0156 u/ul

Tne, 7/22/95 stock = 11.47 u/ul, normalized to Taq p. 127 + 147

11.47 u/ul → 5 ul + 52.35 ul SB → 1 u/ul → 10 ul + 63 ul SB → 0.0156 u/ul

To Pag

Witnessed & Understood by me,	Dat 8/14/95	Invented by	Dat
		Recorded by	8/10/95

g N

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Mg^{2+} 0kcl	48				48	48			48				48											
25kcl		48				48				48				48										
50kcl			48				48				48				48									
85kcl				48				48				48				48								
0kcl																	48			48				
25kcl																		48				48		
50kcl																			48				48	
85kcl																				48				48
1 Mg																								
1.2 Mg																								
1.5 Mg																								

follow blue ink

w/ul Tne 7/22/95	A	2	2	2	2	2															2	2	2	2	
w/ul Tag 5/3/95		2				2				2				2										
Tne																								
56 w/ul Tag					2	2	2	2														2	2	2
Tag			2				2				2			2										
Tne									2	2	2	2												
Tag				2				2				2	2	2	2	2								

To Page No

d & Understood by me,

Polarp

Date 8/14/95

Invented by

Recorded by

Date

Project No._____

162 Book No._____ TITLE _____

From Pag No.__

	25	26	27	25	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
		✓					✓			✓			✓			✓			✓
.5mg oxcl	48				48														
.25xcl		48				48													
50xcl			48			48	48												
55xcl				48		48		48											
50xcl .1mg									48			48			48			48	
.1.2mg										48			48			48			48
1.5mg											48			48			48		
.0156 Tne									2	2	2								
.0156 Taq												2	2	2					
1 Tne	2	2	2	2	—										2	2	2		
1 Taq					2	2	2	2										2	2

1700V, 40mA 12 pm — 12:50 pm

T Pag N

Witn ss d & Underst d by m , D D Polanco

Dat 8/14/95

Inv nt d by

R cord d by Pauline Pond

Dat 8/10/95

Exhibit 155
Appl. No. 09/558,421

164

Project No.

Book No.

TITLE Redid processivity of P 155 expert expand lower [pol] range and correct error in pri / pol from 42 to 1 here)

From Page No.

32p 32 correct • m p19 2 μl
p 155

m p19 0.26 μg/λ 70

70

Vf 70 μl

7.6 pmol circ total
now have
pri / circle = 1

mix A

	70 μl
10 x PCR buffer	175 μl
H_2O	1347.5 μl
50 mM $MgCl_2$	52.5 μl
10 mM dNTP	35
Vf =	1680 μl

32p 33. m p19 ~ 0.22 pmol circ per 50 μl Rxn

(preheat to 70°C
→ 47 μl mix A + 2 μl of pol to start → kill with 25 μl
cycle seq stop sol

same (enz) as p. 155 and 5 more 2 fold dilutions

Tube 15	Tube 14			(units/rxn)			
20 units/50λ rxn	2	1	.5	.25	.125	.063	.031
Taq = 1-15		.0156	.0078	.0038	0.00194	0.000	
		.000484	.000242 ← tube 1				

Tne = 16-29

To Page No.

Witnessed & Understood by me, [signature]
Date 8/14/95
Invented by [signature]
Recorded by
Date 8/11/95

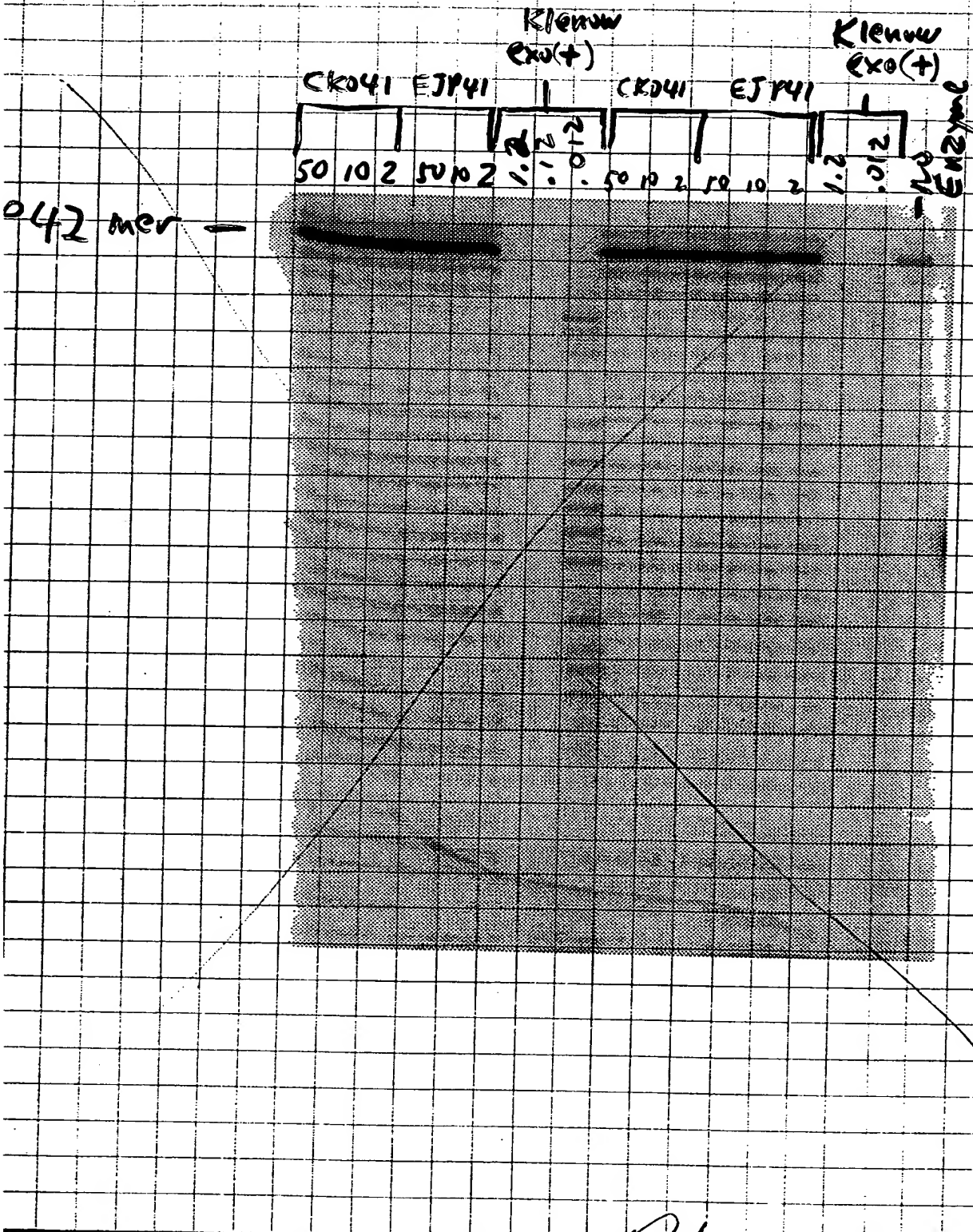
om: Hartman, Chris
o: Lasken, Roger
c: Rashtchian, Ayoub
ubject: Exo minus Klenow Exo(+) Exo(-) Taq
ate: Monday, August 07, 1995 3:40PM
i rity: High

roject N .— Exhibit 156
Book N .— Appl. No. 09/558,421

Reaction Conditions
PCR buffer

ger, The unit values for the bulk exo minus klenow are as follows:

Lot No.	U/ul
CK041	75
EJP41	130



To Page No.

& Understood by me,
Polanp

Date 8/14/95

Invented by

Recorded by

Date 8/14/95

Exhibit **157**
Appl. No. 09/558,421

Project
Book No.________ TITLE Test supermix for [dATP], ~~[dGTP] [dCTP]~~

From Page No.___

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
7 (1) (-dATP)	45 →																							
(2) -dGTP														45 →										
(3) (-dCTP)																								
4 dNTPs	—													—										
2.5 μM		5													5									
5 μM			5													5								
10 μM				5													5							
20 μM					5													5						
40 μM						5													5					
60 μM							5													5				
*Mix B #11								—													—			
2.5									5													5		
5										5													5	
10											5													
20												5												
40													5											
60														5										
H_2O	5							5							5							5		
	50 μl																							

1. assemble on ice
2. put in 9600 4°C
   ramp to 70°C → 45"
   ramp to 4°C

*for B
its 220 μM
(at 1.1x)
∴ for 50 μl
30 μl B
50 μl H_2O
4 = 110 μl

To Page No.

Witnessed & Understood by me, [signature] Date 8/21/95
Invented by [signature] Date 8-15-95
Recorded by

Lane No. 31 32 33 34 35 36 37 38 39 40 41 42

43 is mix (1) ice only
ie no 70°C before EDTA

44 is mix (3) ice only

45 2λ # Rxn # 15 } no TCA
46 2λ # Rxn # 29 }

To Page No.________

Project No._____
Book No._____ TITLE Route P 65

From Page No.__

			pmol
4 dNTP	1	103.00	3.5
	2	264.00	14.8
	3	275.00	15.5
	4	638.00	41
	5	1025.00	68
	6	1248.00	84
	7	2048.00	140
B	8	56.00	0.2
	9	204.00	11
	10	297.00	17
	11	619.00	40
	12	1011.00	67
	13	1430.00	96
	14	1879.00	128
4 dNTP	15	94.00	2.9
	16	488.00	30
	17	653.00	42
	18	1332.00	89
	19	2200.00	150
	20	3902.00	275
	21	5706.00	395
B	22	83.00	2.1
	23	457.00	28
	24	643.00	41
	25	1289.00	86
	26	2218.00	151
	27	4406.00	304
	28	4082.00	282
BKGD	29	53.00	
2 λ	30	57231.00	42.9 cpm/pmol
4 dNTP	1	768.00	8.1
	2	854.00	52
	3	926.00	53
	4	928.00	53
	5	979.00	68
	6	1443.00	198
	7	1721.00	277
B	8	818.00	22
	9	763.00	6.8
	10	971.00	65
	11	931.00	54
	12	1155.00	117
	13	1230.00	138
	14	1473.00	207
	~~15~~	~~16.00~~	
BKGD	16	739.00	not very high ^3H dATP BKGD
2 λ	17	14195.00	

$$\frac{(14155\ \text{cpm})\ (50\ \mu l\ \text{Rxn} + 10\ \mu l\ \text{EDTA})}{(2\ \lambda\ \text{spotted})\ (40000\ \frac{\text{pmol ut}}{\text{Rxn}})} = 10.64\ ^3H\ \text{cpm}$$

To Pag

Witnessed & Und rsto d by m ,	Date	Invented by	Date
		R corded by	

To Page No.

ed & Und rst od by me,	Date	Invented by	Date
		Record d by	

166

Project No.
Book No.
TITLE Determination of how to Kill DNa

From Page No.

DNase I will be used to treat Tne enz prep. If DNA contamin
is the cause (primer dimer) of the smear, than the DNase I treatment may
eliminate the smear. 1st we'll establish how to Kill DNase I, so
after it has been mixed w/ Tne, so it won't be active during R

1 DNase I rxn will be killed w/ EDTA + heat for 5, 10 + 30 mi
A second DNase I rxn will be killed ~~only~~ by heat only for 5, 10, 30
After the Killing treatment, the rxn will be mixed with ØX174 RF
If the DNase I was Killed, the ØX174 won't be degraded, even
after a 3hr incubation.

materials: ØX174, 0.25 ug/ul in 0.1mM EDTA from LTI Lot FA370

DNase I, 1 u/ul in SB = 20mM NaOAc pH 6-5
5mM CaCl2
50% glycerol

25mM EDTA - 50ul 0.5M EDTA pH 8
950ul H_2O

200mM Tris 8.5 (note: the DNase I buffer is 8.4)
200ul 1M Tris 8.5
800ul H_2O

20mM $MgCl_2$ - 200ul 50mM $MgCl_2$
+ 300ul H_2

200mM KCl - ~~200mM~~ 66.7ul 3M KCl
+ 933.3ul H_2O

10x DNase I buffer = 200mM Tris-HCl pH 8.4
LTI 20mM $MgCl_2$
Lot EK2410 500mM KCl - it was a bit bubbly after mixing

0.8% TAE agarose gel w/ EtBr

To Page N

Witnessed & Understood by me, [signature] Date 8/21/95
Invented by Date
Recorded by [signature] 8/15/95

Page N

DNase I rxns: in 9600 PCR tubes

14 ul H_2O
+ 2 ul 10x DNase I buffer
+ 4 ul DNase I, 4 u/ul Cf = 0.2 u/ul
20 ul
Kill with heat + EDTA

(2) 14 ul H_2O
2 ul 10x DNase I buffer
4 ul DNase I Cf = 0.2 u/ul
20 ul - only heat kill this rxn

→ 15' at RT (= 23°C) → during this time 1 ug DNA should be digested

remove 4 ul (= 0.8 units DNase I)
+ 4 ul 0.25 ug/ul ØX174
+ 1.6 ul 10x DNase I buffer
+ 10.4 ul H_2O
20 ul w/ 1 ug

series to see how long it takes active DNase I to degrade 1 ug ØX174

→ immediately remove 4 ul + 1 ul 10x loading dye w/ 100 mM CDTA = 0 Kill time
0 time incubation w/ ØX174

→ 2' ~~later~~ remove 4 ul + 1 ul LD = 0' Kill time 2' w/ ØX174

→ 15' ~~later~~ remove 4 ul + 1 ul LD = 0' Kill time 15' w/ ØX174

→ 1 hr ~~later~~ remove 4 ul + 1 ul LD = 0' Kill time 1 hr w/ ØX174

→ 3 hr remove 4 ul + 1 ul LD = 0' Kill time 3 hr w/ ØX174.

(1) Kill the remaining 16 ul w/ 1.36 ul 25 mM CDTA Cf = 2 mM

(1) & (2) heat to 75°C in 9600

→ 5' of heat kill, remove 4 ul for rxn (1)
+ 4 ul ØX174
+ 4 ul 200 mM KCl Cf = 50 mM, 16 ul
+ 1.6 ul 200 mM Tris 8.5 Cf = 20 mM, 16 ul
+ 2.02 ul 20 mM $MgCl_2$ Cf = ~~2.0~~ 2.02 mM for 20 ul
+ 4.4 ul H_2O
20 ul → 3 hr at ~~25~~ RT °C

To Page No.

sed & Understood by me,	Date	Invented by	Date
DPC	8/21/95	Recorded by Carolyn Combs	8/15/95

From Page No.

I remove 4ul aliquots and treat in the same way after 15' and 30' killing with heat and EDTA

for rxn ②

For the DNase I rxn that was killed by only heat, take 5', 15', and 30' killing time points by removing 4ul of rxn to a tube w/

4ul ΦX174, 0.25 ug/ul
4ul 200mM KCl
1.6ul 200mM Tris 8.5
1.6 ul 20mM $MgCl_2$
4.8 ul H_2O
20ul

- 3hr incubation at RT
- +3ul 10x Loading dye w/ 100
  EDTA
- run 23ul on 0.8% gel

gel order

14 wells

1Kb ladder 10ug	1ug ΦX174	5' heat +EDTA	5' heat only	15' heat +EDTA	15' heat only	30' heat +EDTA	30' heat only	OK:11 0ΦX174	OK:11 2'u ΦX174	OK:11 15' w/ ΦX174	OK:11 1hr w/ ΦX174	OK:11 3hr w/ ΦX174	1Kb ladd

active DNase I incubated w/ 1 ug ΦX174 v
varying amounts of time

To Pag N

Witn ss d & Understood by me, D Polanp

Dat 8/21/95

Inv nt d by

R corded by Kristen Combs

Dat 8/15/95

Exhibit 159
Appl. No. 09/558,421

Project Book No.______ TITLE unit assay for -20°C sample from J8olus and also 1.1X mix sam... on P 34, 52, 60 (unit assay P 1...)

From Page No.__

(note -20°C #11 assayed on P 52)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

-20 #1E 27/1 — 2 2 2
-20 #4E 27/1 — 2 2 2
-20 #7E 27/1 — 2 2 2

1.1X 5-8-95 — 2 2 2
1.1X Field Test ("old" on P34) — 2 2 2

rTaq 1/125 dil 5-30-95 — 2 2 2 2 2

Taq Rxn mix P120,9 — 48µl →

			cpm	relative to Taq u/µl	P122,9 (time 0) u/λ		
-20° 1E	25	5911.00	6531	.03	.037	81%	see P 53 where #11 of 27/1 54% and ... recovery ... #1E, 4 ... show better
	26	6883.00					
	27	6801.00					
-20° 4E	28	5982.00	5649	.026	.029	90%	
	29	5759.00					
	30	5205.00					
-20 7E	31	6079.00	6188	.028	.033	85%	
	32	5062.00					
	33	7422.00					
1.1X 5-8-95	34	4974.00	4773	4773/8686 · .04 = .022	.022/.023 (P34)	96%	
	35	4594.00					
	36	4752.00					
1.1X old Field Test	37	4389.00	4637	4637/8686 = .0213	⇒	93%	
	38	4552.00					
	39	4971.00					
rTaq	40	8930.00	8686 ave	.04 (by definition)			
	41	8601.00					
	42	8299.00					
	43	8980.00					
	44	8616.00					
BKGD	45	78.00					
2X mix	46	100897.00					
	47	102480.00					
	48	102152.00					

To Page

Witnessed & Understood by me, D Polaup — Date 8/21/95
Invented by [signature] — Date 8-16-95
Recorded by

From Page No.___

specific activity of A mix = $\frac{101843 \text{ cpm}}{40{,}000} \left(\frac{50\mu l}{2\mu l}\right) = 63.7$ cpm/pmol nt

pmole = $\frac{cpm}{S.A}\left(\frac{60}{\omega}\right)$(dilution)

8/17/95 cc

SAM	CPM	
1	3072.00	147
2	1533.00	72
3	1127.00	53
4	516.00	24
5	198.00	9.32
6	3423.00	161
7	1581.00	74.5
8	1174.00	55
9	475.00	22.4
10	249.00	11.7
11	3178.00	150
12	2007.00	95
13	2979.00	140
14	2332.00	110
15	2799.00	132
16	2601.00	122
17	2954.00	139
18	2798.00	132
19	3532.00	166
20	1251.00	58.9
21	280.00	13.2
22	3472.00	164
23	2974.00	140
24	2605.00	123

140 pmole $\left(\frac{23.1}{2}\right)$ / 101000 × 3 = 0.494 u/ul (expected 0.

A no killing
A 90° 5'
A 90° 10'
A 90° 30'
A 90° 1hr
B no killing
B 90° 5'
B 90° 10'
B 90° 30'
B 90° 1hr
A 75° 30'
A 75° 1hr
A 75° 2hr
A 75° 4hr
B 75° 30'
B 75° 1hr
B 75° 2hr
B 75° 4hr
Tne, no heat, no EDTA
Tne, 90°, 10'
Tne 90° 1hr
Tne 75° 1hr
Tne 75° 4hr

A = 50 units Tne : 5 units DN
B = 50 units Tne : 1 units D

Nicking assay - after treat
to kill DNase I, the rxn
incubated w/ 1ug φX174
for 3hr at 23°C to ox
any remaining DNase I

note that no untreated
DNA was run as a positi
control for how 1ug looks.
the Tne lanes serve as t
control because Fig 1 shou
Tne has no endonuclease
The 75°C 4hr treatment
EDTA and the lower leve
DNase was best for kil
the low level of DNase degrades 1ug easily

8/17/95 cc

To Pag

Witn ss d & Und rst od by m , Polanp

Date 8/21/95

Inv nt d by

Recorded by Daniel Pomb

Dat 8/17/95

Repeat assay of [illegible]
with -20°C, 4°C side by side

le N	Rxn #	
4°C	1-3	(2pl assayed)
20°C	4-6	
4°C	7-9	
20°C	10-12	
4°C	13-15	
20°C	16-17	
4°C	18-21	
20°C	22-24	
'ase	25	
yeast	26	
nock	27	
nock	28	
Nase	29	
Nase	30	
nock	31	
nock	32	
l stock	33	
l stock	34	
'ul stock	35	
l stock	36	
l stock	37	
l stock	38	

		SAM	CPM1	ave	-20/4C
11E	4	1	7816.00	8440	90%
	4	2	9112.00		
	4	3	8393.00		
	-20	4	7582.00	7558	
	-20	5	7182.00		
	-20	6	7910.00		
4E	4	7	6265.00	6317	102%
	4	8	5771.00		
	4	9	6916.00		
	-20	10	6005.00	6443	
	-20	11	6410.00		
	-20	12	6913.00		
7E	4	13	7478.00	7711	96%
	4	14	7917.00		
	4	15	7738.00		
	-20	16	7016.00	7385	
	-20	17	7461.00		
	-20	18	7679.00		
11E	4	19	5526.00	5211	98%
	4	20	5095.00		
	4	21	5012.00		
	-20	22	5396.00	5100	
	-20	23	5050.00		
	-20	24	4855.00		
	30' Dnase	25	2887.00	2835 ave	70% *
		26	2783.00		
	30' mock	27	2672.00	2814	
		28	2956.00		
	4 hr Dnase	29	3095.00		
		30	3280.00		
	4 hr mock	31	2833.00		
		32	3113.00		
	36 u/ml	33	4234.00		
		34	3968.00		
		35	4606.00		
	5 u/ml	36	4839.00		
		37	3683.00		
		38	4399.00	4041 *	
		39	472.00		
		40	139229.00		

70% 5 u/ul stock dilution
2ul stock 5-7-
19.28ul Taq dil b
21.28ul
↳ 2ul
+45ul Taq dil b
47ul of 0.02 u
* 2835 ÷ 4041 = 70% Dnase
after "30' Dnase" treatment
at 75°C

ons:
and 4hr Tne treated with
eI and mock rxns:
2ul enz, 0.47 u/ul P.175 NGU
45ul Taq dil buffer
47ul of 0.02 u/ul

1ul Tne stock was diluted
0.47 u/ul and then to
u/ul → 2ul 36 u/ul stock of Tne 5-7-95
+12.4ul Taq dil buffer
14.4ul of 5 u/ul
↳ 2ul of 5 u/ul + 19.28ul Taq dil buffer / 21.28ul of .47 u/ul, then diluted
↳ 2ul enz + 45ul Taq

To Page No.

d & Understood by me,	Date	Invented by	Date
Polano	8/21/97	Recorded by	8-17-97

g N.__

atment of Tne with DNase I: 2 rxns of 87.6 ul H_2O
10 ul 10x DNase I buffer
1.39 ul Tne, 36 u/ul, 5-7-95 prep
1 ul DNase I, 1 u/ul
100 ul

test in PCR w/o killed

remove 10ul to 4°C C_f = 0.5 u/ul Tne
0.01 u/ul DNase I
2.5mm $MgCl_2$
1.2% glycerol
0.05mm $CaCl_2$

90ul → 1 rxn 30 15min RT
1 rxn 4hr RT (+15min)

+ 5.74 ul 50mM EDTA C_f = 3mM
new vol = 95.74 ul $C_{f\ Tne}$ = 0.47 u/ul

→ at 75°C for 4hr in 9600 PCR machine
12pm - 4pm, put on ice

trol reactions without DNase I: 2 rxns of 88.6 ul H_2O
10ul 10x DNase I buffer
1.39 ul Tne, 36 u/ul, 5-7-95
100 ul

mock reaction

remove 10ul from each to test in PCR
ie. no killing treatment

90ul → 1 rxn 30 15min RT
1 rxn 4hr RT (+15min)

→ + 5.74 ul 50mM EDTA $C_{f\ Tne}$ = 0.47 u/ul

→ at 75°C for 4hr in 9600
put on ice

To Page No.____

d & Understood by me,	Date	Invented by	Date
Polanco	8/21/95	Recorded by Carolyn Conf	8/17/95

From Page No.__

PCR rxns: 0.5, 1, 2, 4 units Tne
done 8/17/95 long smear conditions and conditions to make specific
- test DNase I treated Tne
- test Tne that has been through a mock DNase I treatr
- test fresh Tne

mix A - to make a long smear
- for 14 rxns 1151.22ul H_2O
28ul 1M Tris 8.5 Cf = 20mM
23.38ul 3M KCl Cf = 50mM
29.4ul 50mM $MgCl_2$ Cf = 1.05mM
28ul 10mM dNTPs Cf = 200uM
1260ul

note: in the rxn with 4 units Tne, the [KCl] will be 55mM

mix B - to make 380bp product
- for 14 rxns 1043.98ul H_2O
28ul 1M Tris 8.5
39.62ul 3M KCl Cf = 85mM
30.4ul 50mM $MgCl_2$ Cf = 1.3mM
28ul 10mM dNTPs Cf = 200uM
28ul 20uM anchor primer Cf = 400n
28ul 20uM 6681 primer Cf = 400nM
28ul 50pg/ul mi3RF Cf = 100pg/rx

dilution of fresh Tne
36.4ul 5-7-95 stock
2ul stock
151.2ul Taq 513
153.2ul of 0.4pu/ul

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
mix A	90	—	—	—	—	—	—	—	—	—	—	—												
mix B													90	—	—	—	—	—	—	—	—	—	—	—
-H_2O	8.9	7.87	5.74	1.49	8.9	7.87	5.74	1.49	8.9	7.87	5.74	1.49	8.9	7.87	5.47	1.49	8.9	7.87	5.47	1.49	8.9	7.87	5.47	1.49
DNase treated Tne	1.1	2.13	4.26	8.51									1.1	2.13	4.26	8.51								
mock treated Tne					1.1	2.13	4.26	8.51									1.1	2.13	4.26	8.51				
fresh Tne									1.1	2.13	4.26	8.51									1.1	2.13	4.26	8.51

100ul rxns started on ice.

To Pag A

Witness d & Understo d by m , DPolans

Date 8/21/95

Invented by

R cord d by Pamela Ponka

Dat 8/18/95

e N __

in Lab is 9600 - 1 min 94°C

35 cycles of { 30 sec 94°C
30 sec 55°C annealing
2 min 72°C elongation
4°C

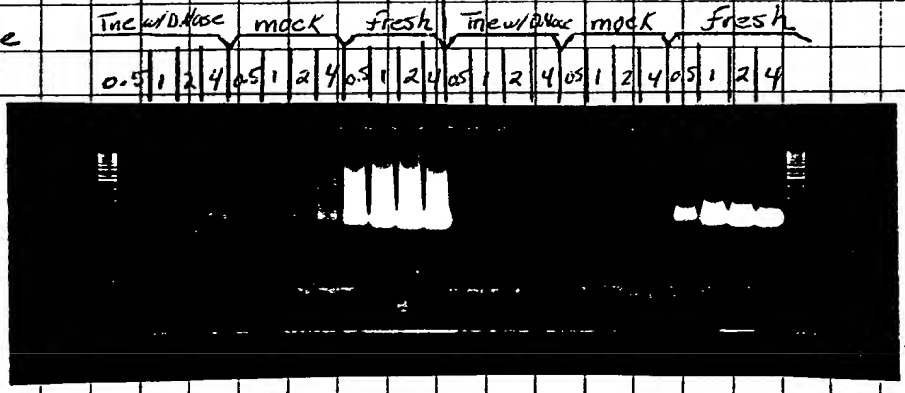
> program 76
method links 71, 75, 74

50 mM KCl
1.05 mM $MgCl_2$

85 mM KCl
1.3 mM $MgCl_2$

Tne treated w/ DNase I for 30' 50u Tne 1 u DNase I

-95 enzyme units



8/18/95 cc

Mock rxn = Tne without DNase I taken through all the DNase I treatment steps

Fresh = untreated Tne used directly from -20°C stock

e: 85 mM KCl did not prevent the small smear from forming

To Page No._____

d & Und rstood by me,	Date	Invented by	Date
Polcyn	8/21/95	Recorded by Caroline Combs	8/18/95

Exhibit **164**
Appl. No. 09/558,421

Projec[t] 32P 33 wrect. mp19 for Klenow exo
'4 Book No.______ TITLE strand displacement assays

From Page No.__

follow P. 138, 9

	lot CK041	lot EJP41	no enzyme	
	①	②	③	4x/
				=0.1 pr
Rxmix (B&D system) * see P.75	147.5 µl	147.5 µl	19.67 µl	0.2
				1750 7
Klenow exo(-) lot				
CK041 P63, 75u/µl dil in Taq SB to 4 u/µl	2.5			20
EJP41 P.63, 130u/µl dil in Taq SB to 4 u/µl		2.5	0.33 µl Taq SB	
	150 µl	150 µl	20 µl of 2.2 µl BJ	

41°C in 9600
remove 20 µl to 2.2 µl 10 x BJ + ~~200~~ 100 mM EDTA — start 1:2
at 1, 2, 5, 30, 90, 2 hr

	well# 1	2-7	8-13
load on 0.8% Agarose	③	①	②

To Page

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	8/21/95	[signature]	8-21-95
		Recorded by	

xperiments on DNase I treated Tne
Mg^{2+} titration & mixing expt

N .__

pose: To determine if & how Tne was damaged by the DNase I
2195 treatment. p.178 mock rxns show low polymerase activity in the PCR, so the 75°C. treatment w/ EDTA affected Tne even if DNase I was not present. A unit assay showed most of the activity was still present (~25% died). Today, we'll add back Mg^{2+} in case there was more free EDTA than we thought. We'll also try poisoning a fresh Tne rxn w/ the treated Tne.

titration: 1.05, 1.3, 2mM MgOAc
smear & product conditions
1 unit DNase treated Tne (30' treatment)
1 unit fresh Tne from 5 u/ul stock

mix for 12 rxn → 24ul 1M Tricine pH9 Cf = 20mM
30ul 2M KOAc Cf = 50mM
24ul 10mM dNTPs Cf = 200uM
882ul H_2O
960ul

B
80ul mix
4.7ul 25mM MgOAc Cf = 1.05mM
2.3ul H_2O
e 90ul/rxn

C
280ul mix
18.2ul 25mM MgOAc
16.8ul H_2O
Cf = 1.3mM

D
280ul mix
+ 28ul 25mM MgOAc
7ul H_2O
Cf = 2mM

mix B + 3 ul Tne treated w/ DNase 30' + 7ul H_2O = 100ul *
mix D + 3ul Fresh Tne Δ + 7 ul H_2O
mix C + 3ul treated Tne + 7ul H_2O
mix C + 3ul Fresh Tne + 7ul H_2O
mix D + 3ul treated Tne + 7ul H_2O
mix D + 3ul Fresh Tne + 7ul H_2O

* Tne at 0.33 u/ul, as determined by unit assay p.73 NB 10 & p.180 NB11

Δ diluted 5 u/ul stock to 0.33 u/ul
5ul of 5 u/ul Tne 5-7-95
70.8ul of Taq SB
75.8ul of 0.33 u/ul Fresh Tne

To Page No. 180

& Und rstood by me,
olano

Date 8/21/95

Invented by

Recorded by

Date 8/21/95

80

Project No.________

Book No.________ TITLE ________

From Page No.__

BAM	CPM1		
1	2952.00	2846	} Tne (DNase Treated 30') dil 1/23.5
2	2739.00		
3	4871.00	4115	} Liz Tne stock 5u/ul 5-7-95 diluted to 0.47 u/ul (same as DNase treated above and then diluted 1/23.5
4	3459.00		

68%

unit assay ~~same~~ same as P73, 10

conclude Tne lost ~70% activity ~~from~~ from killing 4 hr killing of DNase I at 75°C (P175)

product mix for Mg^{2+} titration:

for 12, 100ul rxns = 24 ul 1M Tricine pH 9 Cf = 20mM
51 ul 2M KOAc Cf = 85mM
24 ul 10mM dNTPs Cf = 200uM
24 ul 20uM 6681 primer Cf = 400nM
24 ul 20uM anchor primer Cf = 400nM
24 ul M13RF, 50pg/ul Cf = 100pg/rxn
789 ul H_2O
960 ul

for 3.5 rxns

Ⓔ
280 ul mix
14.7 ul 25mM $MgCl_2$
20.3 ul H_2O Cf = 1.05mM
315 ul
use 90ul/rxn

Ⓕ
280 ul mix
+ 18.2 ul 25mM $MgCl_2$
16.8 ul H_2O
315 ul Cf = 1.3mM

Ⓖ
280 ul mix
+ 28 ul 25mM $MgCl_2$
+ 7 ul H_2O
315 ul Cf = 2mM

To Page No.

Witnessed & Understood by me, DDPolamp | Date 8/28/95 | Invented by | Date 8/21/95
Recorded by [signature]

g No.__

actions → DT = Tne treated w/ DNase for 30' p. 175, 1 unit, 0.33¼l
FT = Fresh untreated Tne diluted to 0.33¼l, 1 unit

smear condition = 50mm KOAc, 20mm Tricine pH 9.0, no template, no primers
product condition = 85mm KOAc, 20mm Tricine pH 9, anchor primer + 6681 primer on m13

[OAc] mM	1.05		1.3		2 mM		1.05		1.3		2	
yme	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT
	1 unit of enz/rxn											
1 %	1	3	4	6	7	9	10	12	13	15	16	18

20ul of each 100ul rxn was run on a 0.8% gel p. 184 →

We had also planned to do rxns with 4 units of the DNaseI treated Tne, but there was not enough of the enz. to set up these rxns

Mg2+ was omitted from rxn by mistake

ning experiment to determine if the DNaseI-treated Tne has a "poisenous" substance in it – mix untreated Tne w/ DNase-treated Tne. smear & product conditions 1 u untreated + 0 treated
0.5 "
1 "
2 "
4 "

mix for smear, 6 rxns = 12ul 1M Tricine pH 9 Cf = 20mM
1.5ul 2M KOAc Cf = 50mM
12ul 10mM dNTPs Cf = 200uM
44.1ul H_2O
480ul → use 80ul/100ul rxn

80ul mix + 3ul fresh Tne (0.33¼l) + 17ul H_2O
80ul mix + 3ul " + 1.52ul treated Tne + 15.48ul H_2O ← see top of page
80ul mix + 3ul " + 3ul " + 14ul H_2O
80ul mix + 3ul " + 6ul " + 11ul H_2O
80ul mix + 3ul " + 12ul + 5ul H_2O

T Page No.__

ed & Understood by me,	Date	Inv nt d by	Date
Polanyi	8/28/95	Recorded by Carolyn Combs	8/21/95

From Page No. 181

2nd try at the mixing expt. - I forgot to add MgOAc to the rxns on

smear buffer: Cf = 20mM Tricine pH 9.0
50 mM KOAc
1.05mM MgOAc
200uM dNTPs

→ no primers or template
mix 1 unit of fresh Tne w/
0, 0.5, 1, 2, 4 units of
mock-treated Tne. p. 17.

for 6 rxns = 12ul 1M Tricine pH 9 ✓
15 ul 2M KOAc ✓
25.2ul 25mM MgOAc ✓
12ul 10mM dNTPs ✓
415.8ul H_2O ✓
480ul → use 80ul mix per 1, 100ul rxn

product buffer: Cf = 20mM Tricine pH 9
85mM KOAc
1.3mM MgOAc
200uM dNTPs
400nM 6681 primer
400nM anchor primer
100 pg/rxn M13mp19 RF template

→ mix 1 unit Fresh Tne w/
0, 0.5, 1, 2, 4 units
of mock treated Tne
p. 1

A unit assay was done on the mock treated Tne on Fri 8/18/95 p. 75 N
We'll assume a concentration of 0.33 u/ul, which was the concentration on 8/18. The Mase treated Tne had not lost any more acti
at 4°C over 3 days, as shown by unit assay on 8/21/95 p. 180. It's likely
the mock didn't loose an

for 6 rxns = 12ul 1M Tricine pH 9 ✓
25.5ul 2M KOAc ✓
31.2ul 25mM MgOAc ✓
12ul 10mM dNTPs ✓
12ul 20uM 6681 ✓
12ul 20uM anchor ✓
12ul 50 pg/ul M13 RF in TE ✓
363.3ul H_2O ✓
480ul, use 80ul mix / 100ul rxn

To Pag N

Witnessed & Understood by m,

Dat 8/28/95

Inv nted by

Dat 8/22/95

R cord d by

g No.

	19	20	21	22	23	24	25	26	27	28	~~29~~
mix ✓	80ul —				1						
ct mix ✓						80ul —				1	
Tne 4ul ✓	3ul (1unit) —									1	
K-treated .33u/ul ✓	0	1:52	3	6	12	0	1:52	3	6	12	
✓	17	15.48	14	11	5	17	15.48	14	11	5	
	100 ul										

~~thod 76, Lab 15 9600~~ Method 103 Lab 16 9600 35 cycles { 94°C 1min; 94°C 30sec; 55°C 30sec; 72°C 2min } 4°C - hold

lution of fresh Tne: 5ul of 54/ul Tne 5-7-95 Lig stock
70.8ul Taq SB
75.8 ul of 0.334/ul fresh Tne

5% TAE gel w/ EtBr, 20ul of each rxn was run on gel

To Page No.

ed & Understood by me,	Date	Invent d by	Date
[signature]	8/28/95	Recorded by Carolyn Combs	8/22/95

Project No. _____

84 Book No. _____ TITLE _____

rom Page No. _____

Results:

Mg^{2+} titration p. 179

DT = Tne treated 30' with DNase I p. 175

FT = fresh, untreated Tne

smear cond: 50mM KOAc inc template/primers

product cond 85mM KOAc

Mixing expt. p. 182

1 unit Fresh Tne + increasing units of mock treated Tne (no DNase)

smear cond: units of

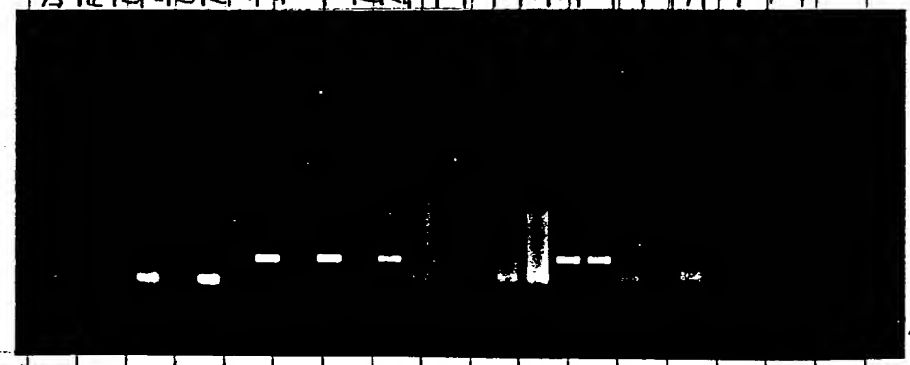
product cond: mock treated

RNase treatment of Tne p. 185

+ = Tne treated w/ RNase

− = Tne not treated w/ RNase

[MgOAc] mM	1.05		1.3		2		1.05		1.3		2		smear		product	
1 unit enz →	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT	0	.5	1	2	4	0	.5	1	2	4	+	−	+	−



cse 8/23/95

Conclusions from Mg^{2+} titration expt:

A unit assay was done on the DNase I treated Tne (see p 180) and fresh Tne. Equal units of the DNase I treated and fresh Tne were used in the Mg^{2+} titration PCR reactions. The treated Tne did not make any specific product, while the Fresh Tne did. We conclude that the 75°C incubation with CDTA (to kill the DNase I) damages Tne. Therefore we do not yet know if treating Tne w/ DNase can eliminate the "bad seed" DNA and prevent a smear.

If there was some residual DNase I activity, the product & smear could be degraded. 2 pieces of data argue against the active DNase explanation. 1) our nicking assay p. 174 shows that 1ug of ΦX174 was not degraded in 3hr and was only nicked a little (~10%) at the DNase I killing treatment

2) in a previous expt (p. 177) the mock-treated Tne showed some low activity in a PCR as the DNase I treated Tne. So, the DNase was not responsible for the low amount of smear products made.

Tne + CDTA died at 94°C, maybe? adding Mg^{2+} back could overcome the damage to Tne p. 177

Further expts to try: → measure thermostability of the 75°C w/ CDTA treated Tne, by cycling the Tne to 94°C before doing the unit assay

→ purify the DNase I-treated Tne away from DNase or "poisen"-gel filtration

To Page No

Witnessed & Understood by me,	Dat	Invented by	Dat
	8/28/95	Recorded by Carolyn Parnell	8/23/95

PCR 1 is 1+2
PCR 2 is 3+4
of 3 → RNase

(#1, 2)

PCR 3 is 5+6
PCR 4 is 7+8

See result on p.184

10ul worth of buffer w/ 100ul worth of dNTP, target, primers

(near) ✓✓ 1 ul Tricine
✓✓ 1.25ul 2m KOAc
✓✓ 2.1 ul 25mm MgOAc
✓✓ 2 ul 10mm dNTP
✓✓ 2ul 6681 → 20uM
✓✓ 2 ul anchor
✓✓ 2 ul m13, 50pg/ul
1 RNaseT1 } both diluted 10 fold in 10mM Tricine
1 RNase A
35.65 ul H_2O
50ul

T1 1460u/ul
A 10mg/mL = 10ug/ul
→ 1u → 40ug RNA/mL
(dil 10x use 1ul)

(#2)

Tne ✓✓ 1 ul Tricine
✓✓ 1.25ul 2m KOAc
✓✓ 2.1ul 25mM MgOAc
→ 2ul Tne (is 5u/l) want 1 unit → dil to 0.5u/l in SB Taq.
1 ul RNase 1
1 ul RNase A } dil 10x in 10mM Tricine
✓✓ ul H_2O
41.65ul
50

15' 37°C → ice → mix → PCR

same w/o RNase (#3, 4)

CSC 8/22/95

Read & Understood by me, Date 8/28/95

Invented by | Date

Recorded by [signature] 8/22/95

From Page No.__

8/22/95 CS

(#5)

expt 3 RNase:

for product

1 ul Tricine 1M
✓✓ 2.13 ul 2M KOAc
✓✓ 2.6 ul 25mM MgOAc
✓✓ 2 ul dNTP 10mM
✓✓ 2 ul 6681 20uM
✓✓ 2 ul anchor 20uM
✓✓ 2 ul m13 50 pg/ul
1 RNase
1 RNase
✓✓ 34.27 H_2O
50 ul

✓✓ 1 ul Tricine
✓✓ 2.13 2M KOAc (#6)
✓✓ 2.6 25mM MgOAc

2 ul Tne 0.5 U/l

✓✓mix 40.27 H_2O
1 RNase
1

✓f same w/o RNase (#7), (#8)

To Pag

Witnessed & Understood by me, Dat 8/28/95 Invented by Dat

Recorded by Paul 8/22/95

e N 184

nclusions from the mixing expt. on p. 184:

The purpose of the mixing expt was to see if 0.5, 1, 2, 4 units of mock reated Tne could poison a PCR with 1 unit of fresh Tne. The mock treated Tne received the 4hr 75°C CDTA treatment but did not contain any DNase I.

The mock-treated Tne did not poison the ability of fresh Tne to make a $\wedge^{10^{-3}}$ smear under the standard smear buffer conditions of 50mM KOAc, 20mM Tricine, 1.65 mM MgOAc. The differences in smear intensity are probably just representative of typical variation in smear intensity. Therefore the mock-treated Tne does not contain a poison that is effective at the levels tested.

The mock-treated Tne also did not poisen fresh Tne's ability to make a specific product (.5u mock w/ 1u fresh still made product. The smears seen with 1, 2, 4u mock pobably ~~are the~~ result from having too many total units. Using more than 1 unit Tne/100ul rxn always results in a smear. ~~The unit of mock was not enough to make a~~ smear. 1 unit mock may not be exactly the same as 1 unit of reated (which doesn't show any PCR activity in the Mg++ titration expt) nd the window for ~~tne~~ activity is probably very narrow. — More ontrols + expt should be done to confirm the absence of a oisen.

nclusions fom RNase expt — Tne treated with RNase A + RNase T1 and then used directly in a PCR. If RNA is the "bad seed" RNase might cure the formation of a smear and increase product yield. The RNase treatment had no effect on either smear formation or product yield. We conclude that RNA is not priming the smear reaction

To Page No.

d & Understood by me,	Date	Invented by	Date
Polanyi	8/28/95		
		Recorded by Evelyn Ennis	8/22/95

From Page No.____

Results:

Mg^{2+} titration p.179

DT = Tne treated 30' with DNase I p.175

FT = fresh, untreated Tne

smear cond: 50mM KOAc no template/primers

product cond 85mM KOAc

Mixing expt. p.182

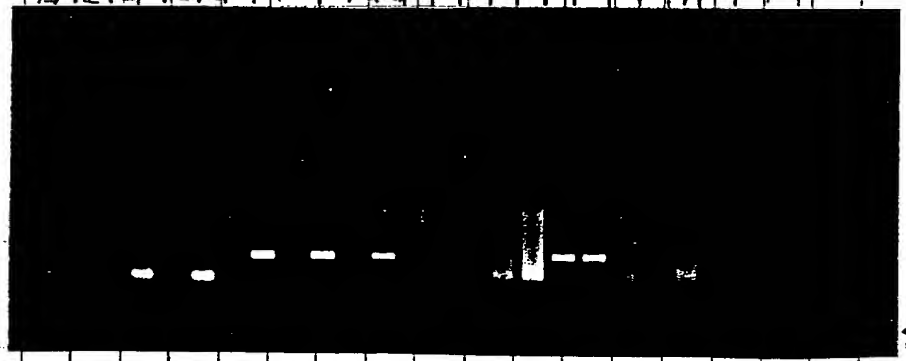
1 unit Fresh Tne + increasing units of mock treated Tne (no DNase)

RNase treatment of Tne p.185

+ = Tne treated w/ RNase

− = Tne not treated w/ RNase

MgOAc mM	1.05		1.3		2		1.05		1.3		2		smear cond units of mock treated					product cond units of mock treated				smear +	smear −	product +	product −
1 unit eng →	1-D	1-F	1.5-D	1.5-F	2-D	2-F	1-D	1-F	1.5-D	1.5-F	2-D	2-F	0	.5	1	2	4	0	.5	1	2	4	+	−	+	−



CSC 8/23/95

Conclusions from Mg^{2+} titration expt:

A unit assay was done on the DNaseI treated Tne (see p. 182) a fresh Tne. Equal units of the DNaseI treated and fresh Tne were used in the Mg^{2+} titration PCR reactions. The treated Tne did not make any specific product, while the Fresh Tne did. We conclude that the 75°C incubation with CDTA (to kill the DNaseI) damag Tne. Therefore we do not yet know if treating Tne w/ DNase can eliminate the "bad seed" DNA and prevent a smear.

If there was some residual DNaseI activity, the product & smear could be degraded. 2 pieces of data argue against the active DNase explanation. 1) our Nicking assay p. 174 shows that 1ug of ΦX174 was no degraded in 3hr and was only nicked a little (~10%) a the DNaseI killing treatment

2) in a previous expt (p. 177) the mock-treated Tne sh the same low activity in a PCR as the DNaseI treated Tne. So, the DNase was not responsible for t low amount of smear products made.

Tne + CDTA died at 94°C, maybe it took adding more Tne to overcome the dominant p. 177

Further expts to try: → measure thermostability of the 75°C w/ CDTA treate Tne, by cycling the Tne to 94°C before doing the unit assay → purify the DNaseI-treated Tne away from DNase or "poisen"-gel

To Page No. ____

Witnessed & Understood by me, [signature]

Dated 8/28/95

Invented by

Recorded by Pamela

Date 8/23/95

ig N .__ 2.2 X reactions, Vf = 0.1 each

Rxmix (B&D)

				Cf at 1X
2 HPO4 pH7.6 P61	~~33~~ µl	16.5 µl	✓	50 mM
dNTPs 10mM	~~13.2~~ µl	6.6	✓	200 µM each
BSA nuclease free acetylated Stratagene cat 300041-51	~~4.4~~ µl	2.2	✓	0.1 mg/ml
50% glycerol	1~~80.84~~ µl	~~90.42~~ 86.9	✓	13.17% ← (includes contributed by Klenow ex.)
1 M MgCl2	~~4.60~~	2.31	✓	7mM
(2 must go in after ...4 is diluted ½ ...l. ppt of Mg PO4)				
33 correct. mp19 ...8,9: 4λ/50µl of Rxn vol ...12 pmol primer ...24 pmol circle ...cle/primer = 2	~~58.8~~ µl	26.4	✓	
H2O	~~360.14~~	183.6 ~~180.1~~	✓	
	Vf = ~~649~~	324.5		

✓
note 5 µl Kl
295 µl Rxn
contributes
(5/300) 50% glycerol
= 0.833%
to Cf
∴ overall Cf
is 14% glycerol
in Rxn

To Page No._____

...sed & Understood by me, [signature] Dat 8/28/95 Invented by [signature] Date [illegible]
Record d by

Exhibit **169**
Appl. No. 09/558,421

Project No.________

32 Book No.________ TITLE Repeat ^{32}P dNTP incorp of Indexed in Tne of P7778 with ΔT

From Page No.__

mix A — for 4 Rxns (Cf 100λ)

		mix A
10 X PCR buffer	✓	40
50 mM MgCl2	✓	56
H2O	✓	260
		356

(Cf = 1150 CPM/pmol 4 / Cf 200 μM each o)

use 95 μl/100 μl Rxn

	①	②	③		
A	89 μl	→		✓	(93.6 u)
Tne C12 36 u/λ	2.6			✓	
Tne PL7-22-95 19 u/λ (see P 127, 11)		5		✓	95 u
rTaq EKBTI dil to 36 u/λ			2.6	✓	93.6
Taq SB	2.4		2.4	✓	
dCTP, dNTP mix*	6	→			
	Vf 100 μl				

heat reaction 1' 94 then lower heat to 7_ then start reaction by addition of ^{32}P dCTP, dNTP mix

*mix
^{32}P dCTP 10 mCi/ml — 16
10 mM 4 dNTPs — 8
Vf = 24

remove 10 μl to 5 μl cycle seq stop solution at 1 2 5 15 30 60 / 90 min

run of 8% PAGE

with ddA, ddT and PFII (Reactions #22, 23, 2_ at top of P 7P) reloaded here as number 22, 23, 24 also.

... is no Enz

To Page N

Witnessed & Understood by me, [signature] | Date 8/28/95 | Invented by [signature] | Date 8-28-95
Recorded by

Exhibit 170
Appl. No. 09/558,421

see P155-157 Ultima and P164 for Tne, Taq

Project No.
Book No.
TITLE Processivity Tne FY vs Ultima

From Page No. 600K11 from P.75

Mix A process (for 37 Rxns, 40 µl/50 µl Rxn)

32P 33mer correct · mp19 circle/primer = 2 ·06 pmol circle A	✓	✓	130 µl	7.8 pmol circles 3.9 pmol primer
H2O	✓	✓	1109.5 µl	for 37 Rxns
10 x PCR buffer	✓	✓	148 (1x at 40 µl V)	use 40 µl/Rxn 50
50 mM MgCl2	✓	✓	55.5 µl (1x at 50 µl Vf)	Cf = 1.5 mM Mg
10 mM dNTPs	✓	✓	37 (1x at 50 µl)	1x at 50 µl Rxn = 2

Vf = 1.48 ml

pol dil (dil in Taq SB)	3.6 U/µl Tne Liz well #	Tne FY 9.4 U/µl	Ultima (5 u/µl)
.00005 U/µl	#1	12	23
.0001	2	13	24
.0002	3	14	25
.0004	4	15	26
.0008	5	16	27
.0016	6	17	28
.0032	7	18	29
.0064	8	19	30
.0128	9	20	31
.0256	10	21	32
.0512	11	22	33

for 40 µl mix = 0.21 pmol
50 µl
~ same as

put 2 µl pol into 8 µl of 1.25 x PCR buffer
preheat to 70°C 1 min in 9600

start with 40 µl of Mix A process (also preincubated to 70)
stop at 2 min with 25 µl cycle seq stop sol.

number "0" is Mix A process 40 µl
Taq SB 2
H2O 8
50 µl
cycle seq stop 25 µl

To Page No.

Witnessed & Understood by me, [signature] Date 9/7/94
Invented by [signature] Date 8-29-94
Recorded by

Exhibit 171
Appl. No. 09/558,421

38

Project N

Book No.______ TITLE Label Fidel pri for incorp opposite Template dU

From Page No.__

Fidel Pri (P54) 100 μM (its a 27mer) 5 μl ✓ (500 pmol total)

γ32P ATP 10 mCi/ml (3.33 μM) 10 ✓ (33 pmol total)

5X Kinase buffer 10 ✓
PNK 10u/λ 1
H2O 24 ✓
50 μl 37°C, 30' → 70°C, 5'

	dT	dU	
	25	25	✓
Fidel Temp (dT)	5		
Fidel Temp dU		5	
P54 100 μM			
10 mM tris pH8	247.78 →	μl	✓
vf	277.8 →	μl	

(~~1000~~ 500 pmol)
(Temp/pri = 2)

Cf = 900 nM prime

↓

90°C 2 min

↓

cool slow

use 5 μl / 50 μl extension reaction for Cf = 90 nM primer

To Page No

Witnessed & Understood by me, [signature] Date 9/7/95

Invented by [signature] Date 8-31-95

Recorded by

Exhibit **172**
Appl. No. 09/558,421

2 **Project No.** ____ **Book No.** ____ **TITLE** ____

From Page No. ____

	(14 Rxns) Cheng I	Taq PCR buffer 14 II		14 Rxns
Same as p. 20 except it has dNTPs at 1mM each at 5X				
5 X Cheng (+dNTPs)	70 µl		✓	its 1.05 mM Mg
10 X PCR buffer		70	✓	
50mM MgCl2		14.7	✓	1.05 mM
10mM dNTPs		14	✓	
Human DNA spleen 80 ng/λ	7 ~~14~~	7 ~~14~~	✓	
10 µM w/ 3267bp i6/i2	14 ~~28~~	14 ~~28~~	✓	
	~~28~~	552.3 ~~28~~		400 nM primer
H_2O	574 ~~532~~	~~545.3~~ ~~503.5~~	✓	
use 47 µl/Rxn 50 µl	672	672		

Cheng #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	(21)	22	23	24	25
I	48 → 12																								
II													48 → 45												

Taq wt (Lig 1-7-95)	5 u/λ	Taq 5B	#
0.25 u/λ 2	↻ 6	6	2
0.5 2	↻ 6	6	2
1 2	2.5	10	2
1.5 2	2	4.67	2
2 2	2	3	2

0.4 / 0.5 µl of 5 u/λ Taq

Taq Δ5 FY	5.4 u/λ	5B		
0.25	5.57	5.5	2	2
0.5	5.5 ↻	5.5	2	2
1	1.2	10.08	2	2
1.5	1	5.27	2	2
2	1.5	5.55	2	2
3	2	4.27	2	2
4	2.5	3.37	2	2
	50 µl			

To Page No

Witnessed & Understood by me, [signature] **Date** 9/7/95 **Invented by** [signature] **Recorded by** **Date** 9-1-95

PCR with OAPDH, glycerol, Chen vs TAC buffer
for Tne wt and ΔSFY
see P 78, 11 for wt conditions

ag No.

94°C 1 min

94°C 30 s
55°C 30 s } 35 cycles
72°C 2 min

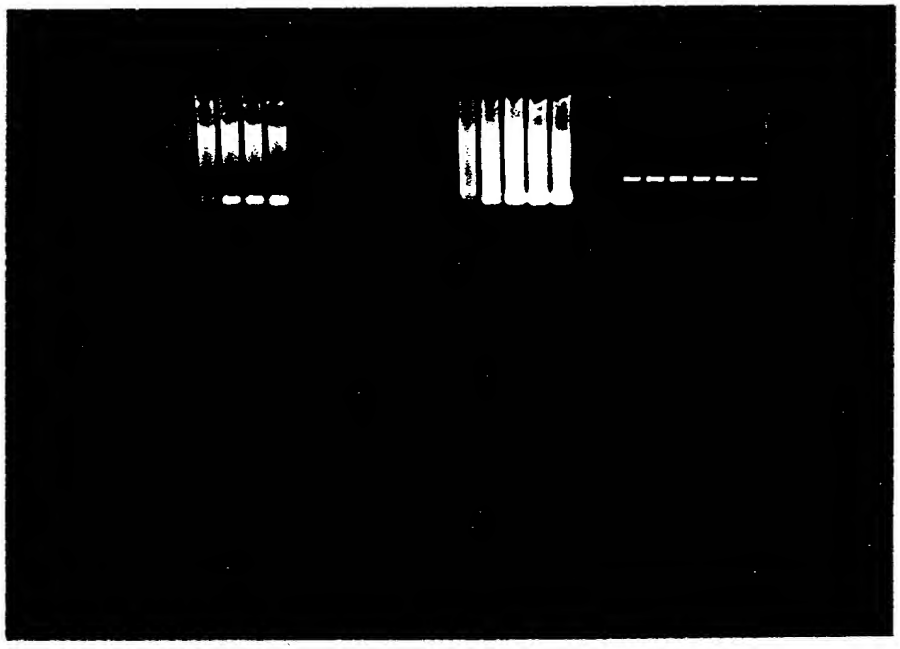
4°C hold (get BJ+EDTA added ≤ 30' after finish in case 3' bpr was probe

Chen buffer | Taq PCR buffer

Tne: wt ΔSFY wt ΔSFY

units 0 1 2 3 0 1 2 3 0 1 2 3 0 1 2 3

2 units Taq pol in Taq PCR buffer



← 267

Result: only get expected 267 bp product for Tne ΔFY if PCR buffer used consistent with lower ionic strength of PCR buffer helping the less processive deleted pol (Chen is 85 mM KOAc compared to 50 mM

To Page No. _____

sed & Understood by m ,	Date	Invented by	Date
Polano	9/7/95	Recorded by	9-14-95

Exhibit 173
Appl. No. 09/558,421

Project PCR with The O5FY

Book No.

TITLE 267bp – 7.5 bp products

From Page No.

Mix A

~~10 min~~ ~~TMAC~~	20	
10x PCR buffer	100	✓ for 20 PCR
~~5x Chem + dNTP~~	~~200 µl~~	✓
50 mM MgCl2	21 µl	✓ (1.05 mM)
Human DNA 80 ng/λ	20 µl	✓✓
H_2O	810.5 ~~251.5~~	✓✓
9.4u/λ The O5FY	8.5 µl	✓✓
	980 µl	

	1	2	3	4	5	6	7	8	9
mix A	49 µl →								
267 bp 16/12 h/g	1								
BDNF 10 µM primers		1							
1.366 Kb primers			1						
2.0				1					
2.82					1				
4.1						1			
5.5							1		
6.166								1	
7.5									1

start 8:40
for 7 min elong need ~ 5 hr
done ~ 2:30

94°C, 1'

94°C 30s
55°C 30s } 35 cycles
72°C 2 min (#1-9) or 7 min (#10-18)
start 11:35 (start 11:25)

need ~ 2½ hr for 2 min e
(done ~ 11:10)

4°C (get BJ + EDTA in ≤ 30' after finish in case 3' exo is a problem

T Pag No

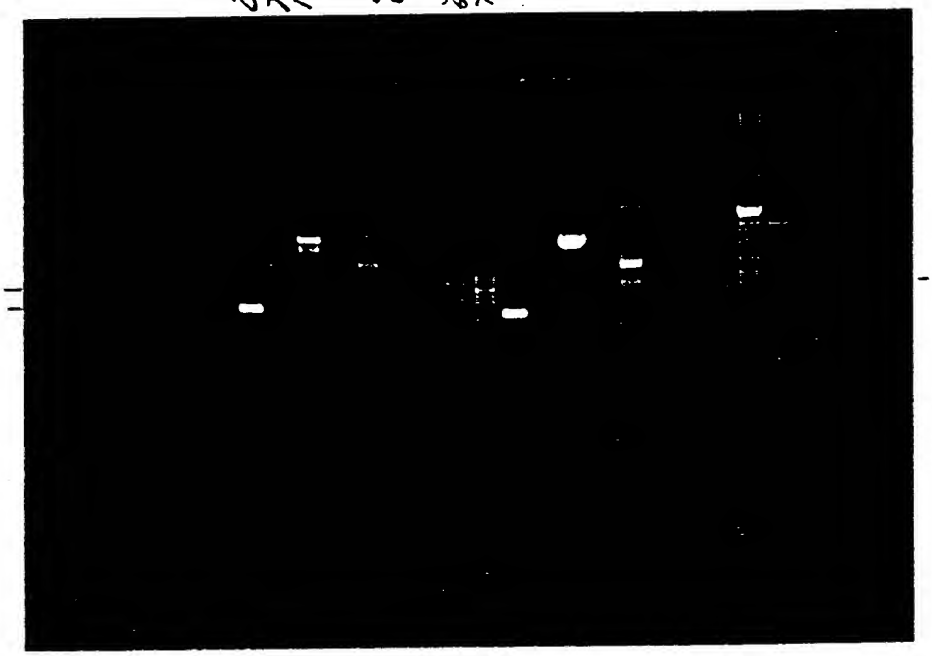
Witnessed & Understood by me, [signature] | Date 9/7/95 | Invented by [signature] | Date 9-6-95

Recorded by

No.

2' elongation

7' elongation



267	+	
800	+	(for 7 min elong
1,36	+	
2	+	(for 7 min
2.8	—	
4.1	—	
5.5	—	
6.16	—	
7.5	—	

To Page No.

ed & Understood by me,

Dat 9/7/95

Invented by

Recorded by

Date 9-8-95

From Page No.

10 x Taq PCR buffer = 200 mM Tris 8.4, 500 mM KCl

10 x ultima buffer is 100 mM Tris pH 8.8, 10 mM KCl

∴ I will try 0 10 25 50 mM KCl cf
(for now I'll stay with 20 mM cf Tris 8.8

	[A]		
1 M Tris pH 8.8	20 µl	✓ (cf = 20 mM)	(for 10 x 1
50 mM $MgCl_2$	21 µl	✓ (cf = 1.05 mM)	
4 dNTPs	25	✓ (200 µM ea)	
Human DNA 80 ng/µl	5 ~~10~~		
9.4 u/µl Taq OSFY	8.5 µl	✓	(4 u/50 µl as per
H_2O	826.5 µl	✓	2 u/100 µl PCR
	940		use 94 µl/100 µl

[A]	① 94 µl →	②	③	④	⑤	⑥	⑦	⑧
KCl	—		—				✓	
200 mM		✓		✓			✓	cf = 1
500 mM			✓		✓		✓	2
1 M				✓		✓	✓	✓
primers								
2.67 kb	16/12 h/q	1 →						
1.36 kb				1 →				
H_2O		✓		✓				
		100 µl						

cycle as per P 94 with 2 min elongation

start 9:40 AM done

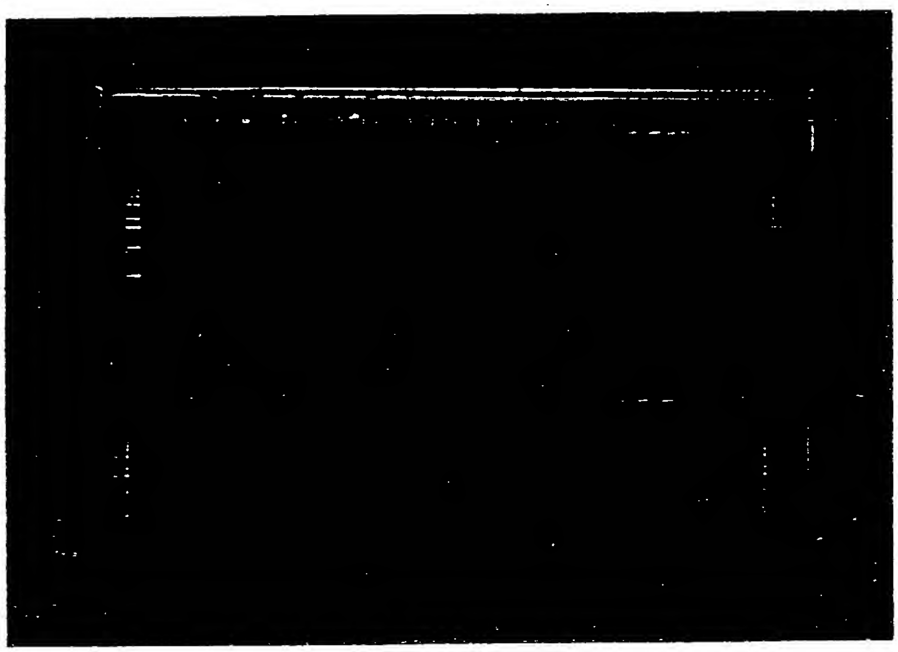
To Pag

Witnessed & Understood by me,	Date	Invented by	Date
	9/7/95	Recorded by	9-7-95

No.__

Results

note 267 bp product does just begin to appear at 35 cycles w/ highest NaCl level of 50 mM



← 267

conclude:
1) higher NaCl₂ helps in contrast to expectation that it would inhibit this destributive form of Tne.
2) target DNA is limiting here since high yields were obtained for 4X more DNA in p 93 and 94

apparently need ≥1 µl of H/S DNA (80ng!) / 50µl Rxn
need only 0.25µl Tne

From Page No.__

They want 10,000 units and same SB as LTI except include 100 mM KCl

Therefore

T4h (formerly thought to be Tf1) 2.5 ml (10825 units total)
4-30-95 (see P8 for units)
4.33 u/µl

* 2M KCl in 50% glycerol and 20 mM Tris pH 7 0.132 ml

Vf = 2.632 ml

final units = 4.1 units/µl

* KCl 0.298 g ✓ (MW = 74.55)
glycerol 1 ml ✓
H_2O to 2 ml ✓
1M Tris pH 7 20 µl ✓

Vf = 2 ml

To Page N

Witnessed & Understood by me,	Date	Invented by	Date
Deborah Polaski	9/9/95	Recorded by	9-9-95